Light- and Electron-microscopic Study of Skin Capillaries of Diabetics

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Except for a few sporadically published reports, investigations of capillary pathology in diabetic patients have in the past been confined to the kidneys and retina. Recently, however, several physiological studies have indicated the possibility of occurrence of diabetic microangiopathy in the distal parts of the extremities. As early as 1930, Starr[1] maintained that he was able to demonstrate a disorder in the minute blood vessels of diabetics. However, he also found that the presence or absence of abnormality of the histamine wheal test was related in part to the diabetic patient's general state of health, and that the results were thus not indicative of disease of the peripheral circulation alone. Handelsmann et al.[2] examined sixteen diabetic subjects, between the ages of forty and sixty-nine, who showed no symptoms of vascular disease in the legs and feet. He measured the change in temperature of the toes after intravenous injection of priscoline and found impaired responses in 40 per cent of the group of asymptomatic patients. Megibow and his co-workers[3] examined forty-seven diabetics, below the age of forty-five, who had no clinically or radiologically demonstrable arteriosclerosis. He studied plethysmographically the change in volume of toes after administration of nitroglycerin and tetraethylammonium bromide, and found evidence of a vascular disorder in fifteen of the patients. Mendlowitz et al.[4] examined thirty-eight diabetics below the age of fifty, by taking calorimetric measurements on the great toe after indirect heating and injection of tetraethylammonium bromide, and found a subnormal blood-flow rate in nine out of the thirty-eight patients. Barany[5] has also made calorimetric investigations after indirect heating. He examined juvenile diabetics under thirty years of age, and found a positive correlation between the duration of diabetes and heat loss, but no relation between the loss of heat and retinopathy. On the other hand Sigroth[6] in a similar investigation on the fingers found a relation between abnormal temperature increase and retinopathy and nephropathy.

All the above-mentioned authors assumed that the deficient response of fingers and toes was attributable mainly to microangiopathy but also advanced the theory that the abnormalities might be the result of autonomic neuropathy. Conversely, Martin[7] proposed the view that the impaired reaction to indirect heating was due solely to autonomic neuropathy and not to angiopathy, since he found a normal response of diabetics to an intravenous injection of priscoline.

In recent years several authors (Bürger[8] and LeCompte[9]) have called attention to the relative lack of histologic data on the minute blood vessels of the extremities in diabetics. Popoff[10] and Masson[11] studied the arteriovenous anastomoses in pulpa from diabetic subjects and found hyalinization of these vessels that did not occur in nondiabetic subjects. Goldenberg et al.[12] investigated the digital arterioles in amputated legs of diabetics and demonstrated endothelial cell proliferation and a PAS-positive, and colloidal-iron-negative, hyalinization of the blood-vessel wall which were absent in arteriosclerotic nondiabetic subjects. Another significant investigation was made by Randerath and Dietzel[13] who found a considerably greater quantity of acid mucopolysaccharides in the intima of minute muscular blood vessels in diabetics than in nondiabetics. In the works of the latter authors, mention was made of the occurrence of capillary hyalinization, but the capillary changes were apparently not studied extensively.

MATERIAL AND RESULTS

Light-microscopic observations. The material of this investigation is composed of samples of skin and subcutis from autopsies and from biopsies on thirty-three patients. In table 1 is given a survey of the clinical material which was divided into three groups. Group A I comprises twelve patients in whom diabetes mellitus was detected before the age of forty. Group A II consists of twelve patients who contracted diabetes after the age of forty. B is the control group comprising nine patients without diabetes. The autopsy material was taken from pulpa of the great toe (and/or thumb) and fixed in 10 per cent formalin and embedded in paraffin wax. The sections were stained with hematoxylin and eosin, according to van Gieson's method, and with the periodic

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FIG. 1. Photomicrograph of capillaries from a cross-sectioned dermal papilla from the great toe of a patient (case no. 1 in table 1) with diabetes mellitus. The capillary walls are greatly thickened by a PAS-positive substance (X). E: erythrocyte, N: nucleus of endothelial cell. PAS and hematoxylin staining. Magnification x 1,000.

acid Schiff method (PAS).

The capillaries of the nondiabetic patients were found to be normal, except in one subject, eighty-one years of age, in whom a slight thickening of the walls with a PAS-positive substance was found.

In many of the diabetics there was a PAS-positive thickening of the capillary walls, notably in the papillae of the dermis and around the sweat glands. The changes were very unevenly distributed in the individual patient, the same tissue section having almost normal capillaries as well as capillaries with pronounced wall thickening. In severe cases the thickness of the capillary walls attained ten times or more than that of the normal capillary (figures 1 and 2). Some capillaries showed a lamellation of the PAS-positive material. The endothelial cells and the number of cells in the regions surrounding the thickened capillaries seemed normal. Ectatic dilatations similar to microaneurysms were not observed, but the PAS-positive substance was frequently considerably more evident on one side than on the other side of a capillary. On the whole the thickening of the walls seemed to continue into the arterioles and venules. The larger the vessels, however, the more difficult it was to distinguish the vascular changes in diabetics from those in nondiabetic subjects.

The degree of thickening of the capillary wall in diabetics is shown in table 1. The extent of thickening observed was graded arbitrarily on a scale of + to ++. In eight patients of group A I the thickening was considered to be moderate to severe. These subjects contracted diabetes before the age of forty, and the disease had been present for periods of fourteen to thirty years. In addition all eight of these patients had nephropathy, and five of them died of uremia. The four additional patients in group A I exhibited minor or no capillary changes. Two of these diabetics had nephropathy but none died of uremia.

In the twelve patients whose diabetes was detected after the age of forty (group A II), no or only slight capillary changes were found. The patients in this group had suffered from diabetes for up to nineteen years.

Uremia alone did not appear to result in thickening of capillary walls since normal capillaries were found in a specimen from a fifty-five-year-old patient with diabetes of five years' duration who died of polycystic kidney disease and uremia. As might be expected, hypertension was often found in the diabetic patients. There was, however, no difference in the occurrence of hypertension between the group of patients with, and those without, capillary hyalinization (table 1).

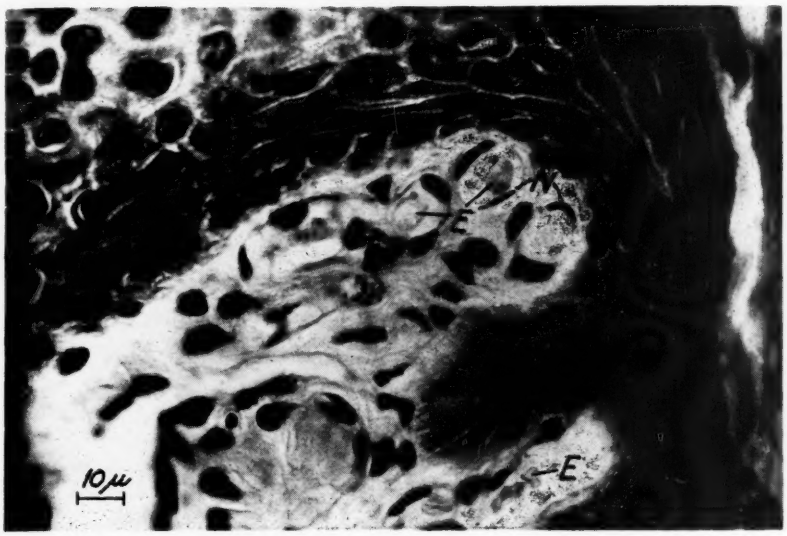

FIG. 2. Photomicrograph of normal capillaries from papillae of the dermis from the pulpa of the great toe. PAS and hematoxylin staining. N: nuclei of endothelium. E: erythrocytes. The basement membrane is indistinct. Magnification x 700.

TABLE 1

Summary of clinical material, incidence and degree of the changes of the capillary wall of pulpa of the great toe: A. Patients with diabetes mellitus

Group I (cases 1 to 12) comprises patients who developed diabetes before the age of forty years.
Group II (cases 13 to 24) developed diabetes after the age of forty.

Case No.	Age at onset	Duration	Retinopathy*	Nephropathy*	Arteriosclerosis	Blood pressure	Cause of death	Degree of thickening of capillary wall*
Group I								
1	31	14	++	++	+	105/60	Nephropathy, uremia	++
2	38	22	++	++	+	145/80	Nephropathy, cerebral thrombosis	++
3	33	30	++	++	+	160/90	Nephropathy, uremia	++
4	16	19	?	++	+	160/95	[Living. Biopsy]	++
5	8	17	++	++	+	165/130	Nephropathy, uremia	++
6	14	22	++	++	+	175/105	Nephropathy, uremia	++
7	12	22	++	++	+	165/100	Nephropathy, uremia	++ or +
8	35	28	?	+	+	145/80	Coronary artery occlusion	++ or +
9	29	18	+	?	?	120/75	Hypoglycemia	+
10	37	21	?	+	+	240/110	Cerebral hemorrhage	(+)
11	39	15	+	+	+	220/110	Cerebral hemorrhage	(+)
12	31	44	÷	÷	(+)	155/105	Embolus of pulmonary artery	÷
Group II								
13	54	17	+	(+)	+	130/70	Cerebral hemorrhage	+
14	65	8	?	?	+	210/100	Ileus	(+)
15	52	5	÷	÷	+	?	Bronchopneumonia, coma	(+)
16	66	10	?	?	+	245/105	Bronchopneumonia	(+)
17	67	19	?	÷	+	95/60	Coronary artery occlusion	(+)
18	72	7	?	÷	+	175/90	Arteriosclerotic heart disease	÷
19	47	2	?	?	+	180/90	Embolus of pulmonary artery	÷
20	55	5	÷	Polycystic kidney	?	135/95	Polycystic kidney, uremia	÷
21	63	13	÷	Pyuria	+	180/90	Bronchopneumonia	÷
22	65	18	?	÷	+	200/140	Cerebral hemorrhage	÷
23	50	5	?	?	+	?	Coronary artery occlusion	÷
24	62	0	?	?	+	140/?	Peritonitis	÷

*Retinopathy, nephropathy and degree of thickening of capillary walls graded on a scale of + to ++, depending upon severity. ? = unknown. ÷ = normal. (+) = slightly abnormal or normal.

TABLE 1 (continued)

Summary of clinical material, incidence and degree of the changes of the capillary wall of pulpa of the great toe: B. patients without diabetes mellitus (controls)

Case No.	Age	Blood pressure	Cause of death	Degree of thickening of capillary wall
25	78	140/80	Pneumonia, arteriosclerosis	÷
26	28	?	Sepsis	
27	50	130/90	Death after heart operation (valvulotomy)	÷
28	59	140/95	Septicemia	÷
29	46	120/85	Septicemia	÷
30	51	140/85	Meningitis	÷
31	70	130/90	Coronary artery occlusion	÷
32	81	130/80	Coronary artery occlusion	+
33	34	125/80	Healthy man, biopsy	÷

Electron-microscopic observations. Electron-microscopic studies were done on pulpa biopsy specimens from two normal men (aged thirty and thirty-four) and from four male diabetics about thirty years old (table 2). Needle biopsies, 2 mm. in diameter, were performed on the pulpa of the fourth finger, and the material was fixed for one hour in Palade's ice-cold buffered 1 per cent osmium tetroxide at *p*H of approximately 7.2, according to Sjöstrand.[14] The biopsy specimens were dehydrated in alcohol and embedded in a mixture of nine parts of butyl metacrylate and one part of methyl metacrylate and polymerized at 48° C. with benzoyl peroxide as activator. Ultra-thin sections of papillae of the dermis were cut on a Sjöstrand ultramicrotome or an LKB ultrotom, and studied in a Philips electron microscope, Type 100 B.

Typical capillaries from the pulpa of a normal subject and a diabetic are shown in figures 3 and 4 respectively. Most of the capillaries from the diabetic patients had far thicker walls than those of corresponding capil-

FIG. 3. Electron micrograph of a part of a normal capillary from a dermal papilla of a finger pulp. L: capillary lumen. N: nucleus of endothelium cell. Mitochondria (M) and numerous vesicles (V) are seen in the cytoplasm. BM: basement membrane. Magnification x 30,500.

FIG. 4. Electron micrograph of a capillary from a dermal papilla of a finger pulp from a patient with diabetes mellitus (case no. IV in table 2). The capillary lumen is closed, forming a v-shaped slit surrounded by three or four endothelium cells. In one of these cells a nucleus (N) is seen; in other cells are mitochondria (M) and an accumulation of vesicles (G) presumably belonging to the Golgi apparatus. Outside the endothelium is a thick, nearly homogeneous deposit enclosing cell portions (X). At F are cross-sectioned collagenous fibrils. Magnification x 13,600.

TABLE 2

Data relating to specimens from diabetic patients examined electron-microscopically

	Age at onset	Duration	Retinopathy*	Nephropathy*	Blood pressure	Arteriosclerosis (X ray legs)
Case IV	12	22	++	++	185/105	+
Case V	9	14	++	++	120/90	÷
Case VII	24	4	÷	÷	110/70	÷
Case VIII	2	29	++	++	140/95	+

*Retinopathy and nephropathy graded on a scale of + to ++, depending on severity. ÷ = normal.

laries from normal skin. The thickening was due primarily to a periendothelial deposition of a material similar in density and structure to the basement membrane. Sometimes the deposited material appeared to be stratified. Usually its structure was compact and dense but occasionally it was nonhomogeneous with a loose, almost spongy texture. It contained no collagenous fibrils, apart from a few in the superficial layers. Frequently flat cytoplasmic processes were embedded in the deposited material, which was thereby divided into two or three layers. Nucleated portions of cells were occasionally embedded in the thickened basement membrane (figure 4).

A more detailed report on the electron-microscopic observations is in preparation.

DISCUSSION

Earlier investigations of vascular function in the fingers and toes of diabetics established a relation between deficient vascular function in these parts of the body and retinopathy and nephropathy. Moreover, it is known that in many cases diabetics have an autonomic neuropathy that often greatly impedes the vascular function (Aagenaes[15]). The present study shows that such patients may also have a severe microangiopathy of the fingers and toes in which the most characteristic morphologic feature is the deposition of a material resembling the basement membrane. This material presumably greatly obstructs normal capillary function and the exchange of chemical substances between blood and tissue. The electron-microscopic observations of the changes within the capillaries of the skin of diabetics are very similar to the glomerular capillary lesions seen in diabetics (Hartmann et al.[16] and Farquhar et al.[17]). The observation of similar abnormalities in these two capillary regions of diabetics again raises the question of a "capillaropathia diabetica universalis," as postulated by Bürger.[8] The studies done by Ashton[18] on a number of blood vessels do not support that hypothesis, however. Considering the diversity in the anatomic structure of the capillaries in different organic systems (Bennett et al.[19]), there is per se no reason to suppose that capillaropathy should be uniform and universal. Capillary abnormalities of the type described may well be localized to certain capillaries of a particular structure and function. Therefore it is of the greatest interest to gain a more comprehensive knowledge of the distribution of diabetic capillaropathy.

Our investigations do not allow us to draw inferences about the chemical nature of the material deposited. Nor do these studies conclusively demonstrate whether the capillary changes are specific for diabetes, or whether they are related to changes characteristic of other diseases, such as arteriosclerosis or hypothyroidism (Baker et al.[20]). The changes are not believed to be related directly to hypertension, since the blood pressure was normal in several of the patients in whom considerable thickening of the capillary walls was observed.

SUMMARY

Twenty-four specimens of pulpa from the toes and fingers of diabetic patients were studied by the light microscope and the structure of capillaries compared with those in similar specimens from nine nondiabetics. A PAS-positive material was found deposited periendothelially in many of the capillaries of the specimens from diabetic subjects. The capillaropathy was particularly pronounced in those patients whose disease had begun before the age of forty and in whom retinopathy and nephropathy were present. In the most severe cases the thickness of the PAS-positive layer was more than ten times the thickness of the normal basement membrane. Electron microscopy of specimens of pulpa from the fingers of four diabetics revealed thickening of the basement membranes of the capillaries which was not present in the control specimens. The similarity of the ultrastructural abnormalities described to those found in glomerular capillaries of diabetics is discussed. The observations reported add support to the concept that diabetes mellitus is associated with a diffuse capillary vascular disease.

SUMMARIO IN INTERLINGUA

Studio a Microscopia de Lumine e Electronic del Capillares Cutanee de Diabeticos

Vinti-quatro specimens de pulpa digital ab le manos e pedes de diabeticos esseva studiate sub le microscopio de lumine, e le structura del capillares esseva comparate con illo in correspondente specimens ab novem nondiabeticos. Un material positive a PAS esseva trovate in deposition periendothelial in multes del capillares de specimens ab diabeticos. Le capillaropathia esseva particularmente pronunciate in le patientes in qui le morbo habeva comenciate ante le etate de 40 annos e in qui retinopathia e nephropathia esseva presente. In le casos le plus sever, le spissitate del strato con positivitate a PAS esseva plus que dece vices le spissitate del normal membrana basilar. Microscopia electronic de specimens de pulpa ab le digitos de mano de quatro diabeticos revelava un spissification del membranas basilar del capillares que non esseva presente in le specimens de controlo. Es discutite le similitude del anormalitates ultrastructural hic describite con illos trovate in le capillares glomerular de diabeticos. Le observationes reportate presta supporto al conception que diabete mellite es associate con un diffuse morbo del vasos capillar.

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On Teaching Diabetes

What Is Needed in the Medical Schools to Improve the Teaching of Diabetes?

We discussed teaching on the inpatient service. It was thought that a consultant or faculty member who devoted most of his time to teaching diabetes could be utilized very well. Inpatient teaching can be done on regular attending rounds, as well as in formal conferences and discussions. The question was brought up whether teaching could be directed to both students and house staff. It was felt that in formal conferences and discussions one could beam his teaching at the proper level.

We then turned our attention again to the importance of integration in teaching. It was felt that integration of the preclinical sciences with clinical teaching was important not only for students but for the faculty as well, because knowledge has become so vast that it has become practically impossible for all to keep abreast of progress at any particular time. We polled the panel on what each individual member felt was the chief problem in his particular institution. Three felt that (lack of) money was the chief problem in the teaching of diabetes. Lack of sufficient faculty was given as the chief problem by two; lack of full-time staff by one; difficulty of staffing and teaching when using part-time faculty by one; better organization of existing facilities by one; utilization of nurses, social service workers, dietitians, and psychiatrists in the teaching program by two; and the importance of integrating within the clinical departments themselves was given by four.

By Benjamin I. Heller, M.D., in
Teaching and Research in Diabetes,
Charles C Thomas, Springfield,
Illinois, p. 29, 1960.

Inborn Errors of Carbohydrate Metabolism

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The concept of "inborn errors of metabolism" was first suggested by Sir Archibald Garrod in 1908. In the Croonian Lectures delivered at the Royal College of Physicians, he suggested that four metabolic disorders — albinism, alkaptonuria, cystinuria, and pentosuria — had certain features in common. In the first place, in all four conditions the onset of the particular abnormality could be dated to the first days or weeks of life, especially when efforts were made to do so. A second characteristic was their familial occurrence in a considerable number of cases. A third feature was that the conditions were relatively benign and compatible with a normal life expectancy. A fourth feature, noted by other clinicians in his day, was the frequency with which these disorders occurred among the offspring of consanguineous marriages.

Half a century later, the original four "inborn errors of metabolism" have increased to well over a hundred, and at least a dozen of these may be regarded as disturbances involving the carbohydrates as shown in table 1. Before going into a detailed discussion of some of the more recent developments in this field, it might be appropriate to consider certain general concepts which cover all carbohydrate disturbances and interpret these in the light of diabetes mellitus, which is the subject of this symposium.

It is interesting to note that six of the inborn errors of carbohydrate metabolism can be identified by the presence of reducing substance in the urine. This means that in each instance wherein the patient is found to have a positive Benedict's urine test, the possibility of the reducing substance being caused by conditions other than diabetes mellitus must be ruled out. This can best be done by separating the sugars, by one dimensional paper chromatography, or by converting the sugar to its osazone and identifying its characteristics. Crude estimates have indicated that essential pentosuria occurs in about one out of 50,000 individuals, essential fructosuria in one out of 120,000 individuals, and renal glycosuria in one out of 100,000 individuals. It would appear that all of these conditions account for somewhat less than 5 per cent of all causes for mellituria in the general population.

It can also be seen that the specific enzyme defect responsible for the block in intermediate carbohydrate metabolism is known in seven of these disorders. In three of these, the defect has been demonstrated in the red blood cell, in three others in the liver, and in the final one, in the muscle. There is considerable indirect evidence to suggest that pentosuria is caused by a deficiency of TPN-xylitol dehydrogenase in the liver. However, the defect has not been demonstrated by direct assay thus far.

Three of these conditions, pentosuria, renal glycosuria and essential fructosuria, are completely benign and

TABLE 1

Inborn errors of carbohydrate metabolism

Name	Reducing substance in urine *Benign*	Enzyme defect
1. Pentosuria	pentose	(TPN xylitol dehydrogenase) (L)
2. Renal glycosuria	glucose	
3. Essential fructosuria	fructose	
	Pathological	
1. Pathological fructosuria	fructose	
2. Galactosemia	galactose	Gal-1-P uridyl transferase (R)
3. Glucose-6-phosphate dehydrogenase		
I. Primaquine sensitivity		G-6-P dehydrogenase (R)
II. Nonspherocytic hemolytic anemia		G-6-P dehydrogenase (R)
4. Glycogen storage diseases		
I. Liver (Von Gierke's)		G-6-P P'tase (L)
II. Heart		
III. Brancher enzyme deficiency		Amylo (1,4→1,6) transglucosidase (L)
IV. Debrancher enzyme deficiency		Amylo-1,6—glucosidase (L)
V. Muscle (McArdle's)		Phosphorylase (M)
5. Diabetes mellitus	glucose	

L = Liver, R = Red cells, M = Muscle.

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compatible with normal life expectancy. Except for the presence of reducing substance in the urine, these conditions would in all probability never have been described in man. However, they do represent variations in normal man, and as such are of considerable interest to the geneticist.

We shall turn now to a more detailed discussion of the pathological conditions which involve carbohydrate metabolism. These may be divided conveniently into five groups: (1) pathological fructosuria, (2) galactosemia, (3) disturbances of glucose-6-phosphate dehydrogenase, (4) glycogen storage diseases, and (5) diabetes mellitus. Since the last area will be covered by the next paper, I shall confine my review to the first four groups of conditions.

PATHOLOGICAL FRUCTOSURIA

Until recently, fructosuria was regarded as a rare error of metabolism characterized by a congenital inability to utilize fructose completely. The condition was perfectly benign, and important only in the differential diagnosis of diabetes mellitus. In 1957, Froesch and his co-workers[1] reported a disorder of fructose metabolism differing from essential fructosuria by its marked intolerance to fructose. The condition was present in seven members of a large family and appeared to be transmitted by an autosomal recessive gene.

In these persons the administration of fructose leads to an excessive and prolonged rise of the fructose concentration in the blood; 10 per cent of the ingested fructose is excreted in the urine, and, simultaneously, with the rise in blood fructose, the blood glucose falls to levels as low as 10 mg. per 100 ml. This severe hypoglycemia lasts for several hours and is frequently associated with nausea, hemorrhagic vomiting, trembling, profuse sweating, and somnolence. After cessation of the acute symptoms, there is a slight and transient icterus, albuminuria, and aminoaciduria.

Froesch and his associates[2] have shown that the erythrocytes of these patients utilize fructose normally, indicating that phosphorylation of fructose to fructose-6-phosphate by the action of hexokinase is normal. During fructose tolerance tests, the fall of serum inorganic phosphorus is fast, exaggerated, and prolonged. This suggests a normal phosphorylation of fructose to fructose-1-phosphate and an exaggerated intracellular accumulation of this ester, resulting from a primary lack of the enzyme 1-phospho fructoaldolase (figure 1). The same authors have shown that in an in vitro system consisting of rat liver homogenate and 1,6-diphospho fructoaldolase, fructose-1-phosphate in concentrations of 1×10^{-2} M will inhibit both fructokinase and 1,6-diphospho fructoaldolase. This inhibition could explain in part the development of fructosemia, hypoglycemia, and hypophosphatemia in patients with this form of hereditary fructosuria.

GALACTOSEMIA

About fifty years ago, the German physician von Reuss reported a case of an eight-month-old infant with marasmus and mellituria. The mellituria disappeared when milk was eliminated from the diet, but the infant died before further tests could be done. Subsequently, other such cases were found. In each instance, the infant appears normal at birth, but after a few days of milk feeding he starts to vomit, becomes lethargic, and fails to gain weight, and enlargement of the liver becomes apparent. Ascites and edema may develop, and if the condition is severe, death occurs due to malnutrition and wasting. Infants who survive are usually malnourished and show signs of dwarfing at two or three months of age, and mental retardation and cataracts may be discerned. In others, cirrhosis of the liver develops months or years after the acute phase of the disease. In all instances, the signs and symptoms regress after deletion of milk and milk products from the diet.

Although it has been known for many years that galactosemia represents a congenital defect of the liver in which the conversion of galactose to glycogen is in some way damaged, it was not until the early 1940's that a series of brilliant studies by LeLoir and his co-workers[3] in Argentina demonstrated the pathway by which galactose was converted to glucose as shown in figure 2. It was found that galactose was converted to glucose-1-phosphate by means of four separate steps, each of which was catalyzed by a specific enzyme.

In 1955, Schwartz et al.[4] in Manchester, England, showed that when milk was given to a galactosemic child, there was an abnormal accumulation of galactose-1-phosphate in the red cells, though this metabolite was not present in normal children. This meant that the block must occur somewhere beyond galactose-1-phosphate, or that galactokinase, the enzyme needed for the first reaction, was present in adequate amounts. In 1956, Kalckar and his co-workers[5] in the United States demonstrated that uridine diphosphogalactose-4-epimerase and uridine diphosphoglucose pyrophosphorylase, the enzymes needed for the third and fourth steps, were present in normal amounts in the erythrocytes of normal children. Nevertheless, galactose-1-phosphate uridyl transferase, the enzyme needed for the second step, was barely detectable in ten galactosemic children. From this they concluded that galactosemia occurs as a result of an enzyme

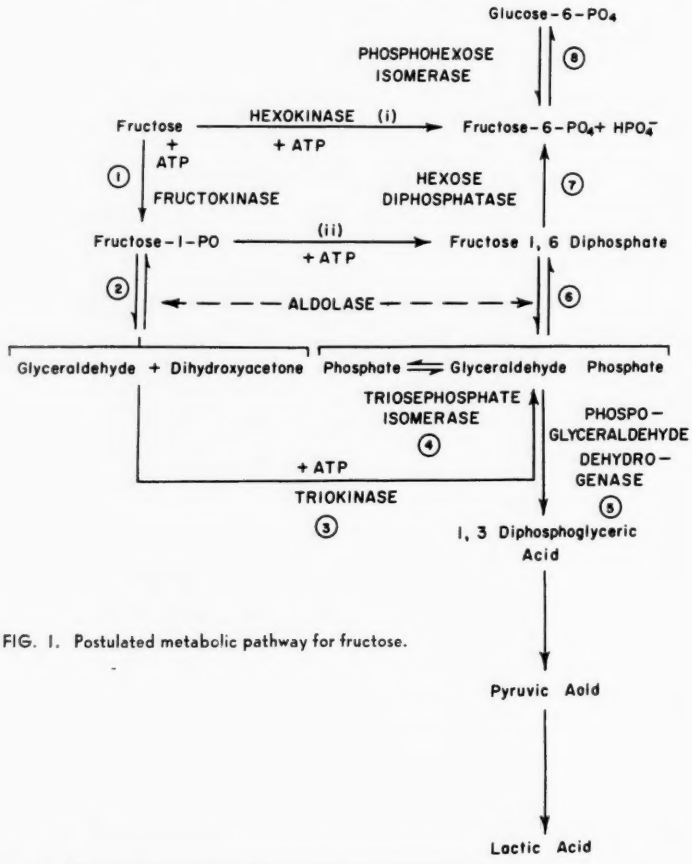

FIG. 1. Postulated metabolic pathway for fructose.

(1) Galactose + ATP GALACTOKINASE Galactose-1-PO_4 + ADP

(2) Galactose-1-PO_4 + Uridine diphosphate glucose P-Gal-URIDYL-TRANSFERASE Glucose-1-PO_4 + Uridine Diphosphate galactose.

(3) Uridine diphosphate galactose URIDINE DIPHOSPHOGALACTOSE-4-EPIMERASE Uridine diphosphate glucose

(4) Uridine disphosphate glucose URIDINE DIPHOSPHOGLUCOSE PYROPHOSPHORYLASE Uridine triphosphate + Glucose-1-PO_4.

FIG. 2. Normal pathway of galactose metabolism in erythrocytes. The enzymes for each step are denoted by capital letters. The metabolic block in galactosemia is marked by an X.

deficiency which causes a metabolic block in step 2 and that all the other properties of metabolism in such patients were normal and intact.

Decrease or absence of galactose-1-phosphate uridyl transferase results in accumulation of galactose-1-phosphate in the red cells, and this in turn contributes to the accumulation of galactose in the blood. The excess galactose can have three kinds of effects as shown in figure 3: (1) galactose and galactose-1-phosphate can accumulate in the tissues and in time are probably responsible for cirrhosis of the liver, cataracts, and renal changes, which are responsible for amino-aciduria; (2) galactose can be excreted in the urine; and (3) galactose and galactose-1-phosphate influence the blood glucose level.

Galactosemia is transmitted by an autosomal recessive gene. The defect has been noted among siblings in about

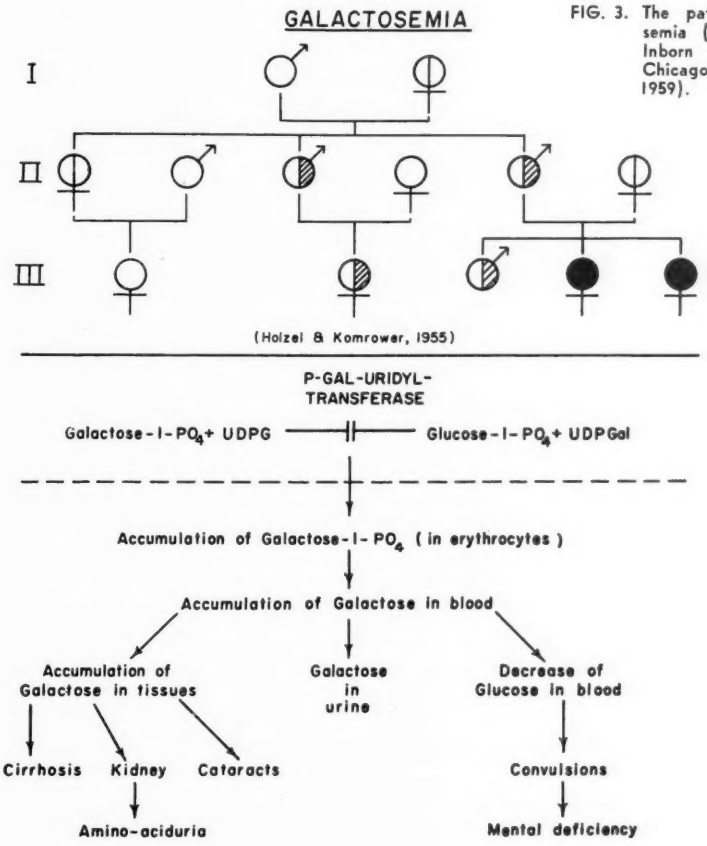

FIG. 3. The pathogenesis of galactosemia (From Hsia, D. Y. Y.: Inborn Errors of Metabolism. Chicago, Year Book Publishers, 1959).

half of the families reported, and it would be expected to occur in one out of four births in such families with a normal distribution. The heterozygous carrier of the abnormal gene may be detected by a decrease of galactose-1-phosphate uridyl transferase among parents of such children. By using the oxygen uptake method, it has been shown that the parents have levels approximately half those seen in normal controls as shown in figure 4.[6]

In our clinic, we have had the opportunity of making detailed observations on fourteen patients with galactosemia.[7] Twelve of the fourteen patients are lively and healthy, but thin. One patient has cirrhosis of the liver. An important and disappointing result of treatment lies in the fact that eight of the fourteen have had visual difficulty (due to cataracts and, in one case, bilateral intraocular hemorrhage), resulting in various blocks in visual activity. Cataract formation has been reported by the fourth day of life, suggesting that mothers of galactosemic children, the heterozygotes of galactosemia, may not be able to metabolize the increased amounts of galactose found in the blood during pregnancy. Transplacental passage of galactose-1-phosphate could result in damage to the fetal lens. Four of our patients, in spite of treatment by four months of age, have residual visual difficulty. If it is assumed that their dietary treatment was lactose- and galactose-free, it is difficult to suggest means of avoiding these complications.

In nine of the fourteen patients, the diagnosis of galactosemia was made by four months of age. Of these, eight have normal intelligence, and the ninth is considerably retarded. Contact with the latter patient has been poor, and it is likely that for economic and other reasons the diet did not remain galactose-free. In five of the fourteen patients, medically supervised diet was not started until twenty-two months of age. Of these, one shows mental retardation. Of the four with normal intelligence, two had restrictive milk intake from late infancy because milk ingestion was observed to produce vomiting and other symptoms, and another received Nutramigen shortly after birth because of consistent diarrhea and vomit-

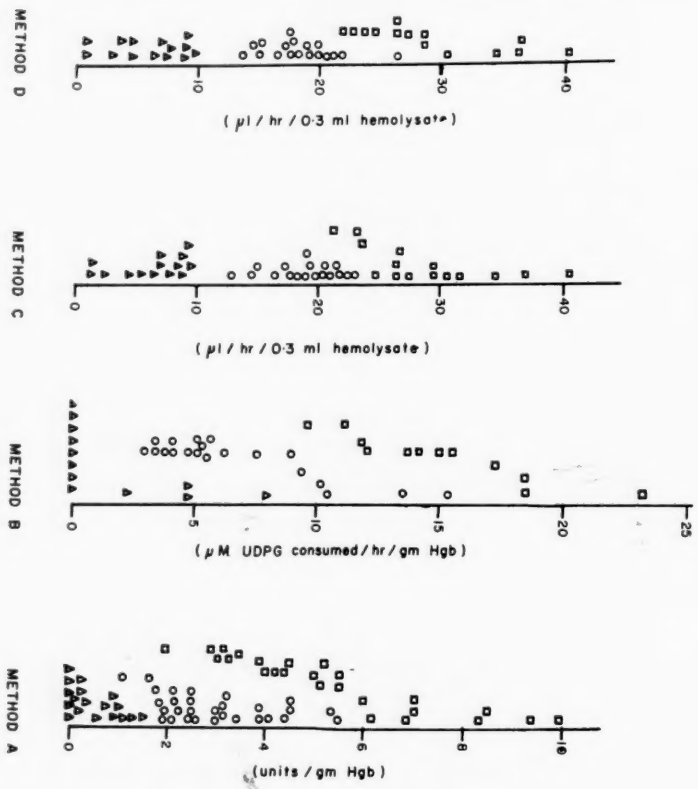

FIG. 4. Galactose-1-phosphate uridyl transferase levels in normal controls (squares), heterozygotes (circles), and homozygotes (triangles) using four different methods of assay. Methods A and B are based upon UDPG consumption and Methods C and D upon oxygen uptake. Details of the methods used are given in reference 6. (From Hsia, D. Y. Y.: Inborn Errors of Metabolism, Am. J. Pub. Health 50:1653, 1960).

ing. The fourth member is interesting because, although he remained undiagnosed and untreated for sixty-two years, he has had a successful life as a business executive.

These cases together with others published in the literature are summarized in table 2. They show that, as in phenylketonuria, adequate dietary control in the early months of life is a factor in the height of intelligence finally obtained. Exceptions do occur, however, and the outcome cannot always be predicted with certainty despite optimal clinical management. Also, it would appear that a greater proportion of untreated patients with galactosemia escape mental deficiency.

Recently, we have encountered an unusual galactosemic family which is worthy of special mention.[8] The pedigree, which is shown in figure 5, indicates that two of the children in the third generation are galactosemic as confirmed by a near-complete absence of galactose-1-phosphate uridyl transferase in their red cells. The parents and all of the mother's brothers and sisters are heterozygotes in view of the fact that the grandfather is a homozygote for the defect as shown by similar enzyme studies. The interesting thing about this family, however, is that all of the heterozygotes in the middle generation on the mother's side spill reducing substance not infrequently. Two of the males were rejected from the armed forces because of persistent reducing substance in the urine. Each of the women runs a 3+ to 4+ sugar in the urine during pregnancy. There is also a history of in-

TABLE 2

Effect of lactose-free diet upon intelligence in forty-six cases of galactosemia

	Intelligence		
	Normal	Moderate retardation	Severe retardation
Adequate dietary control:			
Males	9	3	2
Females	6	1	0
Total	15	4	2
Inadequate dietary control:			
Males	5	5	7
Females	2	5	1
Total	7	10	8

Based upon reported cases and upon reports from the Genetic Clinic.

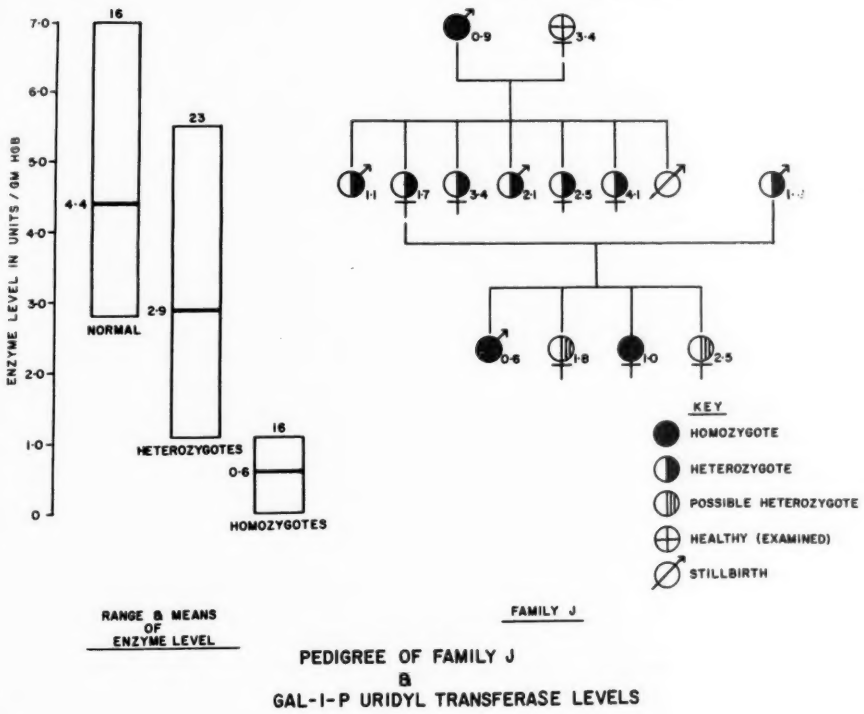

FIG. 5. Pedigree of galactosemic family with reducing substance in urine among heterozygotes. (From Hugh-Jones, K., Newcomb, A., and Hsia, D. Y. Y.: Studies on the Genetic Mechanism of Galactosemia. Reprinted with permission of Arch. Dis. Childhood 35:521, 1960.)

tolerance to milk in some cases. It is interesting to speculate as to whether this represents a combined interaction of the gene for diabetes mellitus with galactosemia, or whether this represents an unusual form of the latter disease genetically. This is currently being investigated. However, it does point out the necessity of making a clear biochemical diagnosis between patients with galactosemia and diabetes mellitus.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

In 1952, Hockwald and his co-workers[9] reported that when primaquine, an antimalarial agent, is given to patients, it induces intravascular hemolysis in about 10 per cent of Negroes, but rarely in Caucasians. It has since been shown that similar hemolytic crises can be induced following the ingestion of fava beans and naphthalene moth balls, and certain drugs such as acetanilid and phenylhydrazine. Laboratory studies have shown that the hemolysis is due to a defect of the red cells themselves and that there is no extracorpuscular defect. Heinz body formation has been noted, and the erythrocytes have been shown to have reduced amounts of glutathione. A deficiency of the enzyme glucose-6-phosphate dehydrogenase in the erythrocytes appears to be the primary defect responsible for this abnormality as shown in figure 6.

The genetic transmission of the defect has been extensively studied by Childs and his co-workers[10] by means of the glutathione stability test. They have shown that male relatives could be divided into reactor and nonreactor groups. On the other hand, the post-incubation glutathione values among family females was continuous, and it appeared that an intermediate group was present. On the basis of these findings, it would appear that the gene for this defect is sex-linked with the variable expressivity. The male homozygote and the female homozygote would be expected to show marked glutathione instability, while the female heterozygote might show all degrees of expression from marked loss of glutathione stability to apparent complete normality of the red cells. We have recently confirmed these findings using a manometric assay for glucose-6-phosphate dehydrogenase as shown in figure 7.[11]

Sensitivity to primaquine is of importance because it is one of the four most common genetic abnormalities known among men. It is estimated that approximately 15 per cent of Negro males and 9 per cent of Negro females are homozygous for this condition. The studies of Motulsky[12] have indicated that the condition is seen most frequently in areas which are endemic for malaria through-

$$\text{(1) Oxidized glutathione} + \text{TPNH} + \text{H}^+ \xrightarrow[\text{Glutathione reductase}]{\text{Oxidized}} \text{2-Glutathione} + \text{TPN}^+$$

$$\text{(2) Glucose-6-PO}_4 + \text{TPN}^+ \xrightarrow[\text{Dehydrogenase}]{\text{Glucose-6-PO}_4 \quad \text{X}} \text{6-Phosphogluconate} + \text{TPNH} + \text{H}^+$$

$$\text{(3) 6-Phosphogluconate} + \text{TPN} \xrightarrow[\text{Dehydrogenase}]{\text{6-Phosphogluconate}} \text{Pentose phosphate} + \text{CO}_2 + \text{TPNH} + \text{H}^+$$

FIG. 6. Site of metabolic block in primaquine sensitivity of erythrocytes.

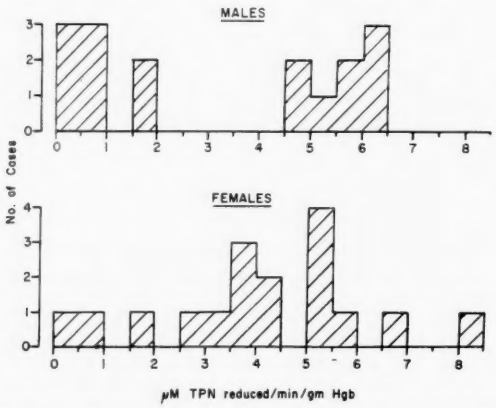

FIG. 7. Distribution of glucose-6-phosphate dehydrogenase activity among primaquine-sensitive families.

out the world. It has been suggested that the individual with primaquine sensitivity is protected from malaria because that parasite requires glutathione in the red cell during its reproductive cycle. This would indicate that the individual who is primaquine-sensitive has an advantage in survival in such areas as Africa, Sardinia, and the Near East, where malaria is prevalent.

Hereditary nonspherocytic hemolytic anemia is characterized by macrocytosis, basophilic stippling, hepatosplenomegaly, and jaundice. Patients with this disease usually come to the attention of physicians because of jaundice and anemia. In the very young age group, the condition is frequently mistaken for erythroblastosis fetalis, except that no blood group incompatibility can be found and the reaction to the Coombs test is negative. In older people, there may be a history of intermittent jaundice, weakness, malaise, or right upper quadrant pain, sometimes accompanied by liver or splenic enlargement.[13]

During the past two years, several groups of investigators[14-16] independently demonstrated a deficiency of glucose-6-phosphate dehydrogenase in the erythrocytes of these patients. From the genetic viewpoint, this was confusing since clinically this group of patients behaved quite differently from the primaquine-sensitive individuals. In the latter, hemolysis only occurs following exposure to one of the primaquine-like agents, whereas in the former, a continuous breakdown of red cells occurs constantly. This confusion has been resolved recently by Kirkman and his co-workers.[17] With observations of kinetics of reactions, they have shown a difference between the enzymic expression of glucose-6-phosphate dehydrogenase seen in one type of deficiency as compared to the other, and that for all practical purposes they represent two independent "inborn errors of metabolism," resulting probably from separate gene defects.

GLYCOGEN STORAGE DISEASES

In the past, it was generally recognized that glycogen storage disease could be found in four forms as shown in figure 8: (1) that due to a deficiency of glucose-6-phosphatase; (2) that of the heart, where no specific enzyme defect has been described so far; (3) that due to

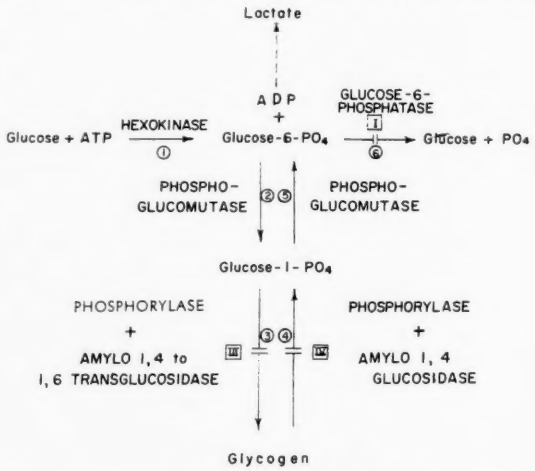

FIG. 8. Normal pathway of glycogen formation and breakdown. A block at site I results in von Gierke's disease; at site III in diffuse glycogenosis with hepatic cirrhosis; and at site IV in glycogen storage disease of liver and muscle.

a deficiency of brancher enzyme (amylo-1,4— 1,6 transglucosidase); and (4) that due to a deficiency of debrancher enzyme (amylo-1,6-glucosidase).

Since the clinical and biochemical aspects of these four conditions have been adequately reviewed,[18] the present discussion will be confined to two new concepts. First, there is increasing evidence that the measurement of phosphorylated intermediates in the blood may be useful in the differential diagnosis between patients with glucose-6-phosphatase deficiency (von Gierke's disease) and other forms of glycogen storage disease,[19] thus making it unnecessary to undertake a liver biopsy to establish the diagnosis. Second, there are now a number of reports[20,21] indicating that siblings affected with glycogen storage disease may have different types of enzymatic defects. While this may occur by coincidence, there are now probably too many examples of this to make them so. One has to consider the possibility of either the interaction of two related genes or the influence of modifying genes to account for these unusual findings.

To the list must now be added a fifth form of glycogen storage disease characterized by a deficiency of phosphorylase.[22-24] In the past, glycogen was believed to be reversibly synthesized and degraded by means of phosphorylase, which is present in two forms, a and b as shown in figure 9. Phosphorylase a, which is the active form of the enzyme, can be readily deactivated to phosphorylase b by PR enzyme (phosphorylase phosphatase). Recently, LeLoir and Cardini[25] have shown that glycogen can be synthesized in a two-step reaction: glucose-1-phosphate can be converted to uridine diphosphate glucose through the action of UDPG pyrophosphorylase; and UDPG can be converted to glycogen in the presence of UDPG glycogen transferase. This synthetic process, unlike that catalyzed by phosphorylase, is not influenced by the concentration of inorganic phosphate and has an equilibrium far to the side of glycogen synthesis.

In 1941, McArdle[26] described a muscle disorder characterized by a rapid exhaustion of otherwise mechanically and physiologically normal muscle. This could be prevented by the intravenous administration of lactate, glucose, or fructose. He suggested that this might result from a metabolic block in the ability to convert glycogen to lactic acid and described the condition as a "myopathy due to a defect in muscle glycogen breakdown." This condition recently has been restudied and muscle from these patients has been found to have considerable amounts of glycogen with normal structure. Furthermore, detailed studies have indicated the presence of normal or nearly normal amounts of phosphoglucomutase, amylo-1,6-glucosidase, UDPG pyrophosphorylase, and UDPG glycogen transferase. However, phosphorylase was found to be markedly deficient. The presence of considerable amounts of glycogen and of UDPG glycogen transferase in the absence of phosphorylase provides strong support for the concept that glycogen is largely synthesized through the UDPG mechanism.

A similar deficiency of phosphorylase in the liver has been reported from Europe.[27,28] No reports of this type of glycogen storage disease of the liver have so far appeared in this country, but the possibility of its occurrence should certainly be considered in view of the deficiency of phosphorylase in the muscle in McArdle's disease.

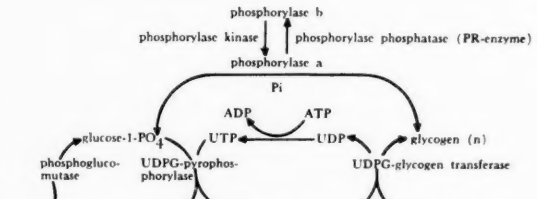

FIG. 9. The synthesis of glycogen.

SUMMARY

The present paper has reviewed some of the recent developments in the inborn errors of carbohydrate metabolism. One must consider these conditions not only in the light of proper differential diagnosis from diabetes mellitus, but also as models by which mechanisms of disease processes can be more clearly understood. It is hoped that it will serve as a fitting introduction to the next paper on the genetics of diabetes mellitus.

SUMMARIO IN INTERLINGUA

Errores Innate del Metabolismo de Hydrato de Carbon

Le presente communication offere un revista de certe recente disveloppamentos in le investigation del innate errores del metabolismo de hydrato de carbon. On debe considerar iste conditiones non solmente in le lumine del correcte diagnose differential ab diabete mellite sed etiam como modellos que permitte un plus lucide comprension del mechanismos pathologic. Es exprimite le spero que iste presentation es un apte introduction pro le communication que seque, le qual es concernite con le genetica de diabete mellite.

ACKNOWLEDGMENT

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Heredity in Diabetes Mellitus

Arthur G. Steinberg, Ph.D., Cleveland

Pincus and White[1] and William Allan[2] were the first to examine by quantitative methods the nature of the genetic factor in the causation of diabetes mellitus. They concluded that susceptibility to diabetes is due to a recessive gene. They found no evidence to cause them to distinguish between the severe, brittle diabetes with onset usually before the age of thirty, and the more benign diabetes which occurs in older patients. Other investigators were reluctant to accept these conclusions, presumably because diabetes is so variable in its clinical expression.

These fine pioneer investigations were followed by a series of studies, too numerous to be reviewed here, leading to several different conclusions.

Some examples of the larger studies will illustrate my point. Cammidge[3,4] scanned the pedigrees of 1,000 diabetic patients and concluded that severe diabetes with early onset (juvenile diabetes) is due to a recessive gene, while mild diabetes with late onset is due to a dominant gene, presumably at a different locus. Harris[5] and Lamy, Frézal, and de Grouchy[6] concluded that early onset diabetes is due to homozygosis for a gene which leads to late onset diabetes when heterozygous. Levit and Pessikova[7] and von Kries[8] believe, on the basis of their studies, that susceptibility to diabetes is due to a dominant gene with incomplete penetrance (i.e., some of those carrying the gene do not develop diabetes). Grunnet,[9] using life table methods, concluded from his study of the families of 261 probands that some of the mild cases of diabetes in elderly individuals are probably nongenetic and that a majority of the severe cases are probably due to a recessive gene. Grunnet fails to state what causes the remainder of the cases. As I have stated elsewhere,[10] the reasons for Grunnet's conclusions are not apparent from his analysis. There have been many other studies of early and late onset diabetes but the conclusions of all of them fall into some one of the above categories. I have reviewed the data from many of these studies in previous reports[10–12] and have there given reasons for concluding that all available data which have been presented in a manner to permit appropriate re-analysis may be explained on the assumption that a recessive gene causes susceptibility to diabetes and cannot be explained by any other simple hypothesis.

I may remark, in passing, that I am unaware of any genetic studies having been done on the J-type of diabetes (onset in young, thin individuals, insensitivity to insulin, and absence of ketosis) reported by Hugh-Jones[13] and by Cosnett.[14] It is to be hoped that this deficit will soon be remedied.

The varied nature of diabetes, the great variation of the age at onset, the profound variation in its severity, its sensitivity to emotional stress and to nutritional status, all contribute to make it a most difficult character for the geneticist to analyze. Indeed, even its relatively great frequency contributes to the complexity of the analysis. If we had very large samples and excellent vital statistics data, we could probably correct for variable age at onset and compute the true expected frequency of diabetes among the sibs of patients from families in which neither, one, or both parents are diabetic. We might also correct for failure to detect diabetes in a prediabetic parent. These corrected figures could be compared with those called for by genetic theory. We do not have the necessary data for such calculations. Alternatively, and preferably, we could make an accurate analysis if we had a method for detecting the prediabetic. Conn and his colleague, Fajans,[15,16] report that the cortisone stressed glucose tolerance test is such a method. Unfortunately, although they apparently have much family data,[16] none of it has been reported in a form which can be analyzed from the genetic viewpoint. Furthermore, there is evidence in papers by Fajans and Conn,[16] by West,[17,18] and by Jackson[19] that prediabetics may have a normal cortisone stressed glucose tolerance test. Drs. I. Zeytin and Priscilla White have kindly permitted me to quote their unpublished data concerning four patients who had a diabetic response to the standard glucose tolerance test and a normal response to a cortisone stressed glucose tolerance test given some time later.

In a paper which appeared in 1959 I wrote, "It would be of interest to know how the frequency of abnormal glucose tolerance tests varies with the age of those

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tested."[10] Such data will permit us to evaluate the effectiveness of the test. If prediabetics are detected with equal facility regardless of how close they are to becoming frank diabetics, the frequency of positive tests should be independent of age. If only those relatively close to becoming diabetic can be detected, the frequency of positive tests should increase with age. West[17,18] and Jackson[19] report that young individuals are less responsive to the glucose tolerance test and to the cortisone stressed glucose tolerance test than are older individuals. Jackson[19] summarizes his conclusions concerning his experience with the cortisone stressed glucose tolerance test as follows:

"1. The mean rise in the tolerance curve after administration of cortisone in apparently normal people over forty-five years of age is higher than in those under forty-five (confirming West's finding[17]).

"2. The mean rise in normal pregnant women is higher than in nonpregnant controls.

"3. A proportion of mild diabetics show no rise at all after cortisone.

"4. A high proportion of patients who are almost certainly prediabetic (on obstetric grounds and/or on account of both parents or an identical twin being diabetic) give negative responses.

"It would therefore appear that although this test may be of some value in indicating the potential diabetic, it frequently fails to do so, whereas in older people or during pregnancy it may appear falsely positive unless the criteria of abnormality are raised."

The available evidence seems to indicate that the cortisone stressed glucose tolerance test may give false positive as well as false negative results.

At present we have no adequate method to correct the family samples for the variable age at onset, nor do we have a method for detecting prediabetics for genetic analysis. Fortunately, we may analyze the data, albeit without the sensitivity we would like, without these desirable aids. It can be shown[11,20] that the relative frequencies of matings with neither, one, and both partners affected among the parents of individuals homozygous for a recessive gene is as p^2: 2pq : q^2, respectively, where q equals the frequency of affected parents. These frequencies are independent of age at onset and of present age. The data from seven samples have been tested in this way. The results are shown in table 1.

We must agree with Pincus and White[1] and with Allan[2] that susceptibility to diabetes mellitus is due to a recessive gene. As I have stated earlier, there is reluctance on the part of many to accept this simple hypothesis, because of the great variability in the expression of the disease. This variation may simply be the variable expression of the effect of the gene causing the disease. I have discussed and illustrated this elsewhere.[22] This implies that diabetes is basically a unitary disease and is in accord with the view held by many physicians. Jackson[19] has stated it this way: "Although the mild obese diabetic and the severe ketosis-prone diabetic are very different clinically, both have the same obstetrical complications and their fetuses are subject to the same embryopathy both in their prediabetic and their overtly diabetic years. The basic similarity between these two types of diabetic is further shown by their similar modes of inheritance, their appearance together in the same family, and the proneness of both types to the same degenerative vascular disease."

While it seems reasonable to conclude that all major studies indicate that susceptibility to diabetes is due to homozygosis for a recessive gene, we have no evidence that susceptibility in every case is due to homozygosis for the same recessive gene. It is possible that in some, susceptibility to diabetes is due to genotype d_1d_1, in others to d_2d_2, etc., where d_1 and d_2 indicate different genes.

TABLE 1

Comparison for seven sets of data of expected numbers of each of three kinds of matings yielding diabetic offspring with the observed numbers. The expected numbers are computed on the assumption of recessive heredity.

No. of diabetic parents	Steinberg and Wilder[11] Obs.	Exp.	Pincus and White[1] Obs.	Exp.	Allan[2] Obs.	Exp.	Harris[5] Obs.	Exp.	Thompson and Watson[21] Obs.	Exp.	von Kries[8] Obs.	Exp.	Lamy et al.[6] Obs.	Exp.
0	1,589	1,588.6	440	440.6	124	122.8	1,124	1,119.1	1,404	1,408.2	1,137	1,135.0	439	437.1
1	370	370.8	80	78.8	17	19.4	109	118.8	223	214.6	160	164.1	58	61.7
2	22	21.6	3	3.6	2	0.8	8	3.1	4	8.2	8	5.9	4	2.2
Total	1,981	1,981.0	523	523.0	143	143.0	1,241	1,241.0	1,631	1,631.0	1,305	1,305.0	501	501.0
ψ^2_1	0.009		0.119		2.109		8.573		2.492		0.885		1.703	
P.	>0.90		>0.70		>0.10		<0.01		>0.10		>0.30		>0.20	

Such a situation is not unprecedented in man. Elliptocytosis,[23] for example, has been shown to be due to at least two different genes.

Unfortunately, no evidence concerning the number of loci leading to susceptibility to diabetes may be derived from the published data, because in collecting the data each family is found via a diabetic child. This means that each parent must carry at least one recessive allele of the same gene present in the homozygous condition in the child, i.e., both carry d_1 or d_2, etc., so that the child may be d_1d_1, d_2d_2, etc.

I have on previous occasions stated that the method most likely to distinguish between the alternatives of a single locus versus two or more loci is to follow the offspring of families in which both parents are diabetic, the families having been selected via the parents without prior knowledge of the condition of the children. If only one locus is concerned, all children should be liable to diabetes and, if followed to a sufficiently advanced age, at least one child in each family should become diabetic. Furthermore, if the number of offspring is large (say 200) it should be possible to estimate the number who are expected to be diabetic. The required data are the distribution of the ages of the offspring and the distribution of the age at onset of diabetes in a large, randomly selected group of diabetics. If two or more loci are involved there should be many families with no diabetic children and the observed frequency of diabetic children should be significantly lower than the computed number.

West[18] has recently published data for ten families which appear to have been ascertained via the parents, although he does not state this explicitly in his paper. His table 1 shows fasting hyperglycemia in one or more children in five of the ten families. One or more children showing an abnormal glucose tolerance test, or an abnormal cortisone stressed glucose tolerance test, or both, were found in four additional families. Thus, nine out of the ten families had at least one child with diabetes or an abnormal glucose tolerance test. In the one family in which this was not so, only one offspring, aged thirty, was examined. He was tested by a prednisone stressed glucose tolerance test, but not by a standard or a cortisone stressed glucose tolerance test. West's data support the view Dr. Russell Wilder and I expressed in 1952,[11] that the vast majority of cases are due to a simple recessive mutation at one locus. However, more data are required and my colleagues and I are collecting them. It would help if others in a position to do so would collect records of families in which both parents are diabetic; *the families being found via the parents, with no previous knowledge of the health of the children.*

I have recently developed equations which may be used with appropriate data to determine if more than one locus is concerned in determining a recessively inherited character, and, if so, to estimate how many loci are involved. The equations are complex and need not be presented here. They will be published in detail elsewhere.[24] The data required to permit their application to diabetes are: (a) the frequency with which diabetes occurs among the first cousins of diabetic patients, both of whose parents are nondiabetic (other families could be used, but they would require a modification of the equations and the collection of additional data); (b) the age distribution of all the first cousins, diabetic and nondiabetic; (c) the distribution of the age at onset of diabetes among a large random sample of diabetics in the general population; (d) an accurate estimate of the number of diabetics in the population; and (e) the age distribution of the population. Such data may be collected in countries with socialized medicine and plans are under way to collect them in Scotland.

If we accept the hypothesis that most, if not all cases of diabetes are due to homozygosis for the same recessive gene, we can estimate the frequency of the gene in the population and, using this estimate, the probability that various relatives of a diabetic patient would be liable to diabetes (Steinberg[12,25]). It is estimated that the gene frequency is about 22 per cent, and that about 5 per cent of our population is genetically liable to diabetes.[11] Since only about 2 per cent of the population is diabetic (detected and undetected, Joslin et al.[26]), it follows that the frequency of the disease may be expected to increase as our population ages. Table 2, from Steinberg,[12] presents a summary of the probability of being genetically liable to diabetes (*dd*) as a function of the relationship to a diabetic patient. The risk runs from less than 20 per cent to 80 per cent. The table does not include the risks incurred when a sib is diabetic because these are clearly 25, 50, and 100 per cent if neither, one, or both parents are diabetic.

While it is of some use to be able to offer probabilities of being liable to diabetes, it would be much more satisfactory to be able to detect the prediabetic; failing that, it would be gratifying to be able to predict ages at onset, and to be able to say that after a given age the risk is essentially nil. The possibility of doing this was raised by Woodyatt and Spetz[27] who concluded from their study of 100 pairs of parent and child diabetics that the average age at onset decreases twenty years per generation, and that a child would rarely, if ever, become diabetic at a greater age than had his parent. This is known as anticipation. Steinberg and Wilder[28] showed that,

TABLE 2

Probability that an individual will be genetically liable to diabetes if he has one or more diabetic relatives*

Probability that person is genetically liable to diabetes†	Diabetic relatives
Up to and including 20 per cent	1. First cousin 2. Uncle or aunt 3. One grandparent 4. Two grandparents (spouses) or one affected parent
30 to 40 per cent	1. Two grandparents (not spouses) 2. One parent and first cousin on nondiabetic parent's side
50 to 80 per cent	1. One parent and a sib of the nondiabetic parent 2. One parent and a parent of the nondiabetic parent 3. One parent, a sib, and a grandparent via the nondiabetic parent

*Relatives refer to parents, grandparents, aunts, uncles, and first cousins.

†Within each group the relatives are listed so as to yield an increasing probability that the person is "dd." Thus, an individual with only a first cousin diabetic (1 of the first group) has the lowest probability of being "dd" while a person with one parent, a sib, and a grandparent via the nondiabetic parent (3 of the last group) has the highest probability of being "dd."

among children of diabetics, the younger age at onset of diabetes is a statistical and not a biological phenomenon. These conclusions were based on an analysis of 200 parent-child pairs for which the ages at onset were known for both parent and child.

More recently I analyzed[10] the data published by Steinberg and Wilder[11] in their paper on the genetics of diabetes. They presented data for 301 parent-child pairs; these included the 200 pairs previously reported.[28] Table 3 presents a summary of these 301 pairs. Onset was in a prior decade of age in 63.4 per cent of the offspring. Hence, as Woodyatt and Spetz[27] reported, prior onset occurs in the majority of the diabetic children of diabetic parents. It may be demonstrated, however, that this frequency of prior onset is precisely what would be expected if we assume that there is no biological relation between the age at onset in parent and child.[28]

The calculations are illustrated in table 4. The expected frequency of children with prior onset for each decade of onset in the parent is obtained by multiplying the value for the proportion of parents with onset in a given decade by the percentage of all diabetics with onset before that decade. Thus, for parents with onset during the fifth decade (forty to forty-nine years), the expected percentage of children with prior onset is $.169 \times .316 \times 100 = 5.3$ per cent. If this operation is repeated for

TABLE 3

Number of patients whose age at onset was in a decade prior to that at onset of the diabetic parent (Data from Steinberg and Wilder[11])

Age of parent at onset	Total no. of parent-child pairs	No. of patients with onset in a prior decade of life
20-29	3	1
30-39	15	5
40-49	51	18
50-59	93	43
60-69	85	71
70-79	41	40
80-89	13	13
Total	301	191

$$\text{Per cent prior onset} = \frac{191}{301} \times 100 = 63.4$$

TABLE 4

Derivation of the expected frequency of prior onset among 301 patients (values are in per cent)

Age, years	Cumulative distribution of age at onset*	Distribution of age at onset among 301 parents	Expected frequency of prior onset in the children
0-9	4.9	—	—
10-19	11.8	—	—
20-29	19.4	1.0	0.1
30-39	31.6	5.0	1.0
40-49	54.1	16.9	5.3
50-59	81.1	30.9	16.7
60-69	96.4	28.2	22.9
70-79	98.8	13.6	13.1
80-89	100.0	4.3	4.2
Total		99.9	63.3

* Based on data of 12,740 patients published by Joslin et al.[20]

the sets of parents with onset in each of the decades, the total expected frequency may be derived. This frequency, 63.3 (table 4), is almost identical with the observed frequency, 63.4 (table 3).

It has been shown that neither the diabetic nor the prediabetic state in the mother influences the age at onset or the probability of occurrence of diabetes in the child (Steinberg and Wilder,[28] Steinberg[10]). Table 5 presents a summary of the data evaluating the possibility of a maternal effect on the age at onset in the child. Table 6 presents the data showing the frequency of diabetes among the sibs of the probands as a function of the sex of the diabetic parent.

In the sample of diabetic parent-child pairs the mean difference between the ages at onset of the parents and the probands is nineteen years. It may be shown by calculations similar to, but more detailed than those illustrated

TABLE 5

Age at onset and frequency of prior onset vs. diabetic parent

Diabetic patients		Diabetic parent Mother (186)	Father (115)
Per cent with prior onset		63.4	63.5
Mean age at onset in years	(a)*	49	48
	(b)*	42	39

*(a) All patients with a diabetic parent.
(b) Patients whose diabetic parents became ill before age fifty: forty-four mothers, twenty-three fathers.

TABLE 6

Frequency of diabetes among the sibs of diabetic patients as a function of the sex of the diabetic parent*

Diabetic parent	Number of patients	Sibs Total	Diabetic No.	Diabetic Per cent
Mother	220	1,000	113	11.3
Father	150	620	72	11.6
Total	370	1,620	185	11.4

* Data from Steinberg and Wilder.[11]

in table 4 that the expected advance in age (assuming independence of age at onset of parent and child) is also nineteen years.[28]

We may conclude that anticipation has no biological significance, that the prediabetic state in the mother does not influence the condition of her child with reference to diabetes, and that we cannot use the parent's age at onset to predict the child's age at onset or the end of the period of risk for the child.

Various investigators[5,6,11] have reported a correlation between the age at onset of diabetes in the proband and in his affected sibs. Harris[5] showed, for his sample, that, assuming no correlation of the age at onset between the proband and the sibs, an apparent spurious correlation of 0.401 would be obtained, because of the curtailing of the distribution due to the lower average age of the sibs of the probands with early onset. The observed correlation (0.695) was, however, significantly greater than the expected (0.401). This led Harris to conclude ". . . that sibs of the early-onset diabetics are very much more likely to develop diabetes in childhood or early adult life than are the sibs of the late-onset diabetics." Steinberg and Wilder[11] also found a significant correlation between the age at onset of the probands and that of their affected sibs, albeit a significantly lower one than that reported by Harris (0.549 vs. 0.695). These authors agreed with Harris that a large portion of the observed correlation was probably spurious because of the similarity in age between the probands and their sibs. They pointed out, however, that much, if not all, of the correlation not accounted for by the distribution of the age of the probands and their sibs may arise ". . . from the peculiarities of establishing the presence of diabetes. For example, if diabetes is diagnosed in a child or young adult living at home, it is probable that others in the home will be examined for diabetes. Therefore, even mild diabetes, which might otherwise continue for a long time before diagnosis, would be detected soon after its onset. Among older persons who had already left home this would be less likely to occur. Other factors are the probable greater accuracy of knowledge of sibs when the patient is young than when the patient is old and the greater similarity of environment between the patients and sibs when the patient is young. The problem requires an intensive investigation of the method of ascertaining the age at onset in the different families. It has characteristics in common with those encountered in 'anticipation' and may very well have no greater biological significance than was shown to be the case for 'anticipation.'" We must conclude that we have no satisfactory way of predicting age at onset. It is worth noting also that a significant correlation of 0.30 (the difference between the spurious and the observed correlations reported by Harris) reduces the total variance by only 9 per cent and hence would hardly be expected to cause sibs of the early-onset diabetics to be "very much" more likely to develop diabetes in childhood or early adult life than are the sibs of the late-onset diabetics.

In summary, it appears that we may be reasonably certain that susceptibility to diabetes mellitus is, with rare exceptions,[10,29] due to homozygosis for a recessive gene; that we have some evidence[18] that only one recessive gene is involved; and that age at onset is not influenced by diabetes in the parent, nor in all probability is it correlated with the age at onset in a diabetic sib.

ADDENDUM

Since this paper was delivered, Dr. Gordon Allen of the National Institute of Mental Health has correctly called my attention to the fact that the method of analysis used by Steinberg and Wilder (1952) is fundamentally a test for random mating (mating without regard to the presence of diabetes) among the parents of the diabetic probands, and not purely a test for the frequency of affected offspring among the different matings as Dahlberg and Hultkranz (1929), who originally derived the method, and as the author, who rederived it independently, believed.

We must conclude, therefore, that simple recessive inheritance, while probable, is not yet proved. The analyses excluding dominant and sex-linked inheritance remain valid.

SUMMARIO IN INTERLINGUA

Hereditate in Diabete Mellite

Il pare satis certe (1) que le susceptibilitate de disveloppar diabete mellite es — con rar exceptiones — le effecto de homozygose pro un gen recessive, (2) que nos es justificate a interpretar le datos a significar que solmente un gen recessive es interessate, a (3) que le etate del patiente al tempore del declaration del morbo non es influentiate per le presentia de diabete in le parente o correlationate con le etate al qual diabete mellite se declara in un fraterno.

ADDENDUM

Depost le presentation de iste discurso, Dr. Gordon Allen del Instituto National de Sanitate Mental ha correctemente portate a mi attention le facto que le methodo de analyse usate per Steinberg e Wilder (1952) es fundamentalmente un test pro le conjugation aleatori (i.e. conjugation sin reguardo al presentia o absentia de diabete) del parte del parentes de probandos diabetic e non purmente un test pro le frequentia de prole afficite de diabete in diverse conjugationes, como Dahlberg e Hultkranz (qui primo—in 1929—derivava le methodo) e como le autor (qui derivava le methodo independentemente) lo credeva.

Per consequente, nos debe concluder que un simple hereditage recessive—ben que probabile—es non ancora provate. Le analyses que exclude le possibilitate de hereditage dominante e de hereditage ligate al sexo remane valide.

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Experiences with Phenformin

A Two-year Study

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The place of oral therapy in diabetes is gradually being defined. Initial impressions of the usefulness of the sulfonylureas have been largely sustained,[1,2] although the incidence of secondary failure has reduced their value to some extent. The place of the biguanides is less certain. In contrast to the sulfonylureas, the biguanides have a hypoglycemic effect in insulin-dependent or juvenile as well as adult diabetes and there was some hope initially that they would have a place in the therapy of all types.[3,4]

It was in an effort to assess this place that the present study began in February, 1958, employing phenethylbiguanide (phenformin or DBI).

MATERIALS AND METHODS

An attempt was made to control all new cases of diabetes presenting at the Strathclyde Hospital Diabetic Clinic on phenformin alone. This amounted to thirty-seven cases. Strict criteria were used in deciding the need for a hypoglycemic agent. These consisted of a preliminary attempt to control the disease by diet alone; failure to do this was judged by obtaining postprandial blood sugar levels in excess of 200 mg. per 100 ml.,[5] two hours after a main meal, on several occasions. A placebo tablet test was then used and only after this was shown to have failed was the drug given. In more acute cases, where the severity of the disease demanded more active treatment, the patient was usually admitted to hospital for a short preliminary period.

Phenformin was also used in sixteen patients who had shown either primary or secondary failure to the sulfonylurea group of drugs.

Sixty-four patients who had previously been controlled with insulin were included in the study when it was found that insulin by itself was unable to give satisfactory control as judged by the blood and urinary sugar levels or by the frequency of hypoglycemic reactions. Early in the study it was hoped that the drug would replace insulin, but latterly, for reasons which will be discussed later, this aim was confined to stable cases.

Having satisfied ourselves as to the need for the drug, the cases were allotted to the following groups according to the age and mode of onset of the disease.

1. *Adult Stable* — Gradual onset over forty years without ketonuria.
2. *Juvenile Stable* — Gradual onset under forty years without ketonuria.
3. *Adult Unstable* — Acute onset over forty years with ketonuria in the absence of a history of obesity.
4. *Juvenile* — Acute onset under forty years with ketonuria.

The carbohydrate intake was limited to 170 gm. per day except where a heavy occupation necessitated an increase. Where, in unstable cases, ketonuria persisted despite the use of the drug, supplementary insulin was given until the ketonuria was controlled and thereafter, where possible, was withdrawn. Obese patients were not included in the series.

Phenformin tablets, 25 mg., were given twice daily after food and the dose gradually advanced until satisfactory control was obtained or the patient became intolerant of a further increase. The mean dose for the series was 116 mg./day; only three patients required more than 200 mg./day. In general, increasing the dose above 150 mg./day did not increase the hypoglycemic effect. Of the three cases in which 200 mg./day was exceeded, two proved resistant to a maximum dose of 300 mg./day, although they tolerated it well. The remaining patient had diarrhea at 250 mg./day.

RESULTS

Before considering results certain terms necessary to their expression will be defined.

(a) *Early failure.* This includes those patients in whom phenformin failed to show a useful hypoglycemic action (primary failure) or in whom the drug was

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withdrawn because of side effects before an assessment of its potency was possible. In the case of primary failures the drug was given for a minimum period of two months.

(b) *Late failure.* This term is used where withdrawal was necessary after a period of complete or partial success (see below) on account of late side effects or from secondary failure of its hypoglycemic action. The latter group (viz. secondary failure) requires some explanation, since the phenomenon has not been described hitherto in patients treated with phenformin. The term is used only where, after a period during which the drug was shown to have a useful hypoglycemic effect, the blood sugar rose in the absence of any other factor such as infection or undue weight gain (indicating neglect of diet) and where increasing the dose of DBI failed to restore control. In those subjects in whom the drug was used with insulin, the insulin requirements rose and in relevant instances reached the dose of insulin required prior to treatment with DBI. The objection that the disease may have become more severe, explaining the course of events, is refuted by the unchanged insulin requirement when the drug was finally withdrawn.

(c) *Temporary success.* This term is partly synonymous with late failure since it indicates a temporary period of success, in this series varying from two to sixteen months, which was terminated by late failure or by the addition of insulin, thus converting a "temporary success" into a "partial success." Although a temporary period of successful treatment measured in months may appear insignificant in a disease lasting for years, we feel that it is noteworthy in unstable diabetes, since hitherto no drug apart from insulin has achieved even temporary control.

(d) *Partial success.* This refers to patients in whom phenformin was used to supplement insulin. By "success" is meant that insulin dosage was reduced significantly (at least by one third) and the resulting control was better than that obtained with insulin alone as measured by freedom from hypoglycemic reactions and closer blood sugar control.

(e) *Successes.* This includes only patients in whom control by accepted criteria was obtained with phenformin alone. These criteria were as follows: (1) Morning and evening urine specimens contained little or no sugar by Clinitest methods. (2) Postprandial blood sugar two hours after a main meal was consistently less than 140 mg. per 100 ml. (3) Weight was constant. (4) Symptoms of diabetes were absent.

In some cases the aim of treatement was to supplement insulin rather than to replace it completely. A comparison of the success rates alone would be misleading and we have therefore indicated the number of cases in which phenformin had a useful effect by a summation of the successes and partial successes. The temporary success group is not included as it is of interest rather than of immediate value.

The results are summarized in tables 1 and 2. The over-all success rate seems low in that only forty-two of 117 cases (37 per cent) were successfully treated and the drug was useful in 53 per cent. This, however, conceals the more impressive results in the adult stable group where the success rate was 63 per cent and in 72 per cent phenformin was of value. No juvenile unstable cases were successfully treated and a useful effect was obtained in only 27 per cent. The stable juvenile and unstable adult groups lie between these major divisions in their success and partial success rates.

The effect of previous treatment on the success rate is important as can be seen from table 2. Thus the drug was of value in 68 per cent of the previously untreated cases and sulfonylurea failures but only 40 per cent of those previously treated with insulin. The actual success rates show greater differences but as stated above these are not strictly comparable since the aim of treatment was not the same in all groups.

TABLE 1

Results of treatment in 117 patients

	Adult stable		Juvenile stable		Adult unstable		Juvenile unstable		Total	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Total treated	56		17		7		37		117	
Early failures	6	11	3	17	2	29	9	24	20	17
a. No hypoglycemic action	6		1		2		5		14	
b. Early side effects	—		2		—		4*		6	
Late failures	10	18	6	35	2	29	18	48	36	31
a. Gastrointestinal side effects	7		4		—		2		13	
b. Ketoacidosis	2		—		1		8		11	
c. Secondary failure	1		2		1		8		12	
Partial success	5	9	2	12	2	29	10	27	19	16
Success	35	63	6	35	1	14	—	—	42	37
Total successes		72		47		43		27		53

*Includes one case in which ketoacidosis appeared with normoglycemia within one week of start of therapy.

TABLE 2

Comparison of results in relation to previous therapy

	Previous treatment					
	None		Insulin		Sulfonylurea failures	
	No.	Per cent	No.	Per cent	No.	Per cent
Total	37		64		16	
Early failures	1	3	17	27	2	13
a. No hypoglycemic effect	—		12		2	
b. Early side effects	1		5		—	
Late failures	11	30	22	35	3	19
a. Gastrointestinal side effects	2		9		1	
b. Ketoacidosis	5		6		1	
c. Secondary failure	4		7		1	
Partial success	1	3	17	27	1	6
Success	24	65	8	13	10	62
Total successes		68		40		68

From a consideration of the above results it would be expected that the drug would be most useful in adult stable cases that had not been treated previously; in fact this is so. Twenty-two successes occurred in twenty-six cases (85 per cent) and the remaining four cases were temporary successes for a mean of eight months.

A hypoglycemic effect was demonstrated in all previously untreated patients, but only in 82 per cent of those previously treated with insulin and 85 per cent in sulfonylurea failures.

Secondary failure occurred in twelve patients (10 per cent) and eight of these were juveniles (22 per cent of thirty-seven patients) and only one adult stable case (see table 1). Withdrawal of the drug made no change in the course of the control and reintroduction of the drug in four patients did not alter insulin requirements. This we feel confirms the loss of hypoglycemic activity in a drug which was demonstrated to be active initially.

TOXIC EFFECTS

A. *Deaths.* Five patients died while under treatment with DBI. Three of these were from unrelated causes (two from congestive cardiac failure, one from carcinoma of stomach with metastases). Of the remaining two deaths, one has been reported elsewhere.[6] This was of a young man admitted in coma with a blood sugar of 208 mg. per 100 ml. and an alkali reserve of 3.1 mEq./L. He was being treated with 50 mg. phenformin thrice daily and 12 units IZS (Lente) daily. Death occurred a few hours after admission.

The remaining fatality occurred in a stable juvenile man of thirty-seven who had been successfully controlled by phenformin for eleven months when he developed a febrile illness. He was treated with an antibacterial sulfonamide; when nausea appeared he ceased to take all tablets and was admitted five days later having had neither antibiotic nor hypoglycemic agent of any kind. He died almost immediately and at autopsy was found to have lobar pneumonia with abscess formation. Postmortem blood sugar was 1,060 mg. per 100 ml.

B. *Ketoacidosis.* This feature has been observed before[7] and we have reported upon it elsewhere.[8] Clinically there appears to be a disparity between the hypoglycemic and the antiketotic effects, the former being much more powerful than the latter if indeed the latter exists. Although ketoacidosis necessitated withdrawal of the drug in only eleven cases (9 per cent), ketonuria occurred in thirty-four cases in the series, eleven in adult stable cases (20 per cent) and seventeen in juvenile unstable cases (46 per cent), the remaining six being in the other groups. A total of thirteen cases had an alkali reserve of less than 20 mEq./L. and the blood sugars varied from 55 to 280 mg. per 100 ml. (figure 1). In seven cases ketones disappeared when the carbohydrate element of the diet was increased and in eleven cases when the dose of phenformin was reduced. In the remainder, it was necessary to increase or add insulin, or withdraw the drug. In juvenile unstable cases this was a frequent cause of failure after a period of adequate control

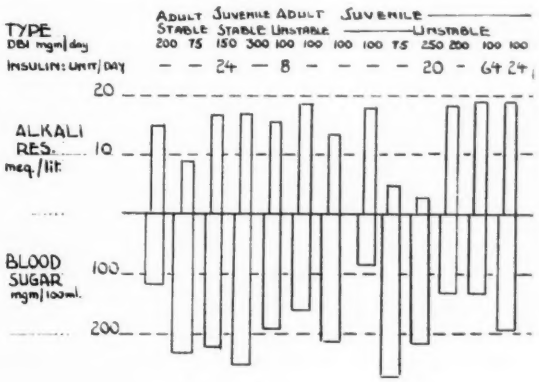

FIG. 1. Alkali reserve and blood sugar levels in thirteen patients with normoglycemia and an alkali reserve below 20 mEq./L.

(mean seven months); its appearance was sudden and unpredictable. It was only responsible for withdrawal of the drug in two adult cases. The mean dose of DBI of patients in all groups who showed ketosis was 146 mg./day, compared with the over-all mean of 117 mg.

C. *Alimentary Symptoms.* Evidence of gastrointestinal irritation either in the form of nausea or diarrhea occurred in forty-one cases (35 per cent). In twenty-three of these it was transitory and responded either to a reduction in dose or persistence with the drug. In five, DBI was not tolerated from the start of treatment and in the remaining thirteen nausea developed late and was persistent enough to necessitate the withdrawal of the drug. Like others we used larger initial doses early in the series and when small initial doses and smaller increments were used nausea became less common. The absolute dose bore no relation to the incidence of nausea (table 3). The mean dose of the patients with alimentary symptoms was 118 mg./day compared with 116 mg./day for the whole series.

TABLE 3
Incidence of alimentary side effects in relation to daily dose of phenformin

Dose of phenformin (mg./day)	25	50	75	100	125	150	200	>200
Number of cases	2	13	28	32	2	21	16	3
Number of cases with alimentary side effects	0	6	7	11	1	9	6	1
Percentage of patients with alimentary side effects	0	46	25	34	50	47	38	33

DISCUSSION

While we have confirmed the hypoglycemic activity of phenformin in all types of diabetes, the experiences recorded above have led us to the view that its most useful application is to the stable adult group not previously treated with insulin. While this has already been shown to apply to the sulfonylureas, our over-all success rate of 85 per cent in this group compares favorably with these drugs. The use of DBI can thus be recommended as a safe and effective measure for the restricted proportion of this group which requires a hypoglycemic agent. Where insulin has been used previously in patients of this type, the success rate has been strikingly lower (six of nineteen = 31 per cent). This has also been shown in sulfonylurea cases.

The successes in juvenile stable cases have been frequent enough to make this a method of treatment to be considered. While it may be thought that this is an ill-defined group, we found that the absence of ketonuria in some cases of gradual onset allowed us to make this distinction with some confidence. The convenience of an oral drug in place of insulin is in our opinion valuable enough to warrant the extra attention required, possibly with stabilization in hospital.

In our experience failure of response to one sulfonylurea (primary or secondary) is usually a failure to this group of compounds. This together with the high incidence of secondary failures makes phenformin a valuable alternative to insulin in an increasing number of patients. While our series was not large, 62 per cent were successfully treated (table 2) for a mean of ten months.

Initially, optimistic reports led us to try phenformin in juvenile cases. In our first case its use alone controlled the diabetes well for a period of sixteen months; in this instance as in the other ten juvenile unstable patients in whom the drug was used alone, secondary failure of its hypoglycemic activity or ketoacidosis obliged us ultimately to replace the drug or supplement it with insulin. The average period of "Temporary Success" was 6.0 months (two to sixteen) and in each case ketonuria appeared with normal blood sugar levels but in only three cases did this force us to withdraw the drug in that it was not abolished by increase of carbohydrate intake or reduction of phenformin dosage. However, good control for long periods may be terminated suddenly and unexpectedly by ketoacidosis; when it occurs with hyperglycemia it is due to secondary failure; with normoglycemia however, we consider it to be a toxic effect of the drug. In our opinion the drug should not be used alone in the treatment of these cases as the period of control offered is negligible in a disease of many years' duration. An additional factor is the tendency of juvenile patients on DBI alone to lose weight, in spite of satisfactory urinary and blood sugar levels. Eight of the eleven cases of this type lost or (in the case of children) failed to gain weight.

The place of supplementary treatment has been disputed and is admittedly hard to define. There is no doubt that the dose of insulin is reduced and in our experience this gave more stable control with relative freedom from hypoglycemia. We regard this as a useful function of the drug but it must be used with caution. Even a substantial dose of insulin does not protect a patient from ketoacidosis. In twelve of twenty-eight juvenile cases, ketonuria occurred in the presence of normal blood sugar levels; in five of these patients this caused withdrawal of the drug. Of the twenty-eight, eight had an alkali reserve of less than 20 mEq./L. The patient whose death is referred to above was having supplementary insulin.

It is unfortunate that the hypoglycemic activity of phenformin was initially considered to the exclusion of

the antiketotic effect. Ketoacidosis can appear insidiously and unexpectedly and this may be attributed to the fact that many clinics examine for ketones only in the presence of glycosuria. It is our belief that this must have led to the dangerous phenomenon of ketosis with normoglycemia being overlooked. In the juvenile patient this may appear after a prolonged period of good control; at this point attendance at a clinic may have been less frequent and in our experience the phenomenon was not always heralded by malaise. It is possible that unusual exertion may precipitate a crisis of this type and evidence exists which substantiates this view.[9]

The significance of ketoacidosis with relation to the mode of action of the drug is not certain, but it is evident that phenformin is not strictly insulin-like or insulin-potentiating. This is confirmed by the high lactic acid levels produced by the drug.[9]

In some of our cases, the use of phenformin provoked ketosis in patients who had not previously shown ketonuria and this in circumstances where starvation acidosis was disproved by normoglycemia or mild hyperglycemia; indeed the withdrawal of phenformin without substitution with insulin caused the ketones to disappear. It could accordingly be argued that the drug antagonizes the antiketotic effect of insulin. This was strikingly illustrated by two adult stable patients in whom replacement of 14 units of insulin by phenformin led to serious acidosis with alkali reserves of 15.5 and 9 mEq./L. This has made us unwilling to substitute even low doses of insulin with DBI in a single step; indeed we prefer hospital admission.

In our view the symptoms of early nausea, malaise and diarrhea have been important only in a minor way and when the dose of the drug is reduced or when the current dose is maintained, it disappears. The late appearance of nausea in the well-controlled patient was our major cause of failure in this group.

SUMMARY

Two years' experience of treatment of 117 cases of diabetes with phenformin is reviewed.

The drug is strikingly successful in the adult patient who has not been treated with insulin previously, frequently successful in the juvenile stable case and in the patient in whom the sulfonylureas have failed. It may also have a place as a supplement to insulin in stabilizing the poorly controlled juvenile unstable type of case.

The danger of ketoacidosis is serious and demands caution in the use of the drug in all insulin-dependent cases. Supplementary insulin does not prevent this phenomenon. The exact mechanism of production of ketoacidosis is uncertain but it is suggested that in those cases receiving supplementary insulin the antiketotic effect of insulin is antagonized.

SUMMARIO IN INTERLINGUA

Experientias con Phenformina: Un Studio de Duo Annos

Es presentate un revista del experientias de duo annos de therapia a phenformina con un casuistica de 117 diabeticos.

Le droga es frappantemente successose in le patiente adulte qui non ha recipite ulle previe therapia a insulina; illo succede frequentemente in stabile casos juvenil e in patientes in qui le sulfonylureas esseva tentate in vano. Illo ha possibilemente etiam un placia in le tractamento de mal stabilisate casos juvenil del typo instabile in le quales illo pote funger como supplemento de insulina.

Le risco de cetoacidosis es serie, e multe circumspection es necessari in le uso del droga in omne casos a dependentia de insulina. Supplementos de insulina non preveni iste phenomeno. Le precise mechanismo responsabile pro le production de cetoacidosis non es clar, sed le these es proponite que in patientes recipiente insulina supplementari, le effecto anticetotic del insulina es antagonisate.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the help obtained in this study from Dr. A. C. Aitkenhead, in whose unit the study took place. The staff of Strathclyde Hospital Diabetic Clinic and Law Hospital Biochemistry Department have given us much assistance and for this we are grateful. The drug was supplied by the U.S. Vitamin & Pharmaceutical Corporation.

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A Comparison of Urine and Blood Tests in Diabetes Detection

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Both urine and blood testing procedures have been employed in diabetes detection. Knowledge of the sensitivity and specificity of such tests under conditions of random times in relation to food eaten or after specified periods of time after eating is extremely valuable in providing information regarding the yield of new cases of diabetes which may be expected, and the retest load which may be anticipated under various conditions of testing. A number of evaluations of this type have already been made.[1,2,3]

The newer enzyme tests which measure true glucose in urine have proved as sensitive as older tests which measure total reducing substances, and this is achieved without sacrifice of specificity.[4] These enzyme tests afford the simplest and most rapid method ever devised for testing large numbers of urine specimens within a short time. Nevertheless, the sensitivity and the specificity of such tests, as of any test upon urine, are influenced by the renal threshold for glucose, which when low operates to increase the number of false positive tests (renal glycosuria) encountered, or when high operates to reduce the number of true positive tests, in relation to the true number of diabetics tested.

Testing procedures for blood sugar are not subject to the above limitation, but they are not devoid of other problems. Some commonly employed blood sugar tests (Folin-Wu) measure many reducing substances other than glucose normally present in human plasma although tests are available (Somogyi-Nelson) which, for practical purposes, determine "true" glucose only. Both types of blood tests generally entail more technical manipulation and are more time consuming than urine tests, and for this reason have not been as widely used in routine testing for diabetes. Also, since such tests give quantitative results, a predetermined level must be established for screening purposes, and the results in terms of sensitivity and specificity will obviously depend upon the levels chosen. Although ability to control the screening level possesses many advantages, chosen levels which achieve high sensitivity often pose a problem of low specificity, and vice versa. Urine tests generally do not present such a decision problem, since any degree of positivity, other than trace results for certain tests, such as Benedict's, is usually designated as a positive test.

Some of the major objections to the use of blood sugar determinations in diabetes detection have been overcome by a mechanical laboratory apparatus known as the Hewson Clinitron which uses tablet reagents that are dispensed automatically, and requires only a given amount of either finger-tip or venous blood in 5 ml. of water. This instrument can complete as many tests in an hour as are ordinarily performed by a trained laboratory technician in an eight-hour day. Since the initial cost of this instrument is high, it is adapted mainly to mass testing programs. However, a less expensive instrument, the Glover-Edwards Glucose Test Kit, which employs the same tablet reagents, has recently become available* and may be suitable for small scale or office testing if its performance approaches that already demonstrated for the Clinitron. With either of these instruments testing can be performed at predetermined levels of 130 mg., 160 mg., or 180 mg. of "true" glucose, corresponding to the Somogyi-Nelson method. Once the level for performance of the test has been selected, this method renders reports which are either "positive" or "negative." Information is available regarding sensitivity and specificity achieved at each of these levels under specific circumstances, for example, in relation to time when last food was eaten.[5]

The present study was undertaken to compare the "relative sensitivity" and "relative specificity" of finger-tip blood tests using the Clinitron set to test at 130 mg., with four commonly employed urine tests: Benedict's qualitative test, Tes-Tape,† Clinistix,‡ and the Clinitest.‡ Although a testing level of 160 mg., or 180 mg., can be employed with the Clinitron, as indicated above, we have found that the 130 mg. level produces

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†Eli Lilly and Company, Indianapolis, Indiana.
‡Ames Company, Inc., Elkhart, Indiana.

superior results,[6] and have therefore limited this comparison with urine tests to results achieved with the Clinitron operated at this level.

It should be pointed out that our data do not give information regarding "absolute" sensitivity and specificity performance for these tests. This would have required that every person tested, regardless of whether the screening test was "positive" or "negative," be subjected to definitive procedures either to rule out or rule in a diagnosis of diabetes. This would have made available a more accurate denominator of "true" diabetics for determination of sensitivity, and of "true" nondiabetics for determination of specificity. Strictly speaking, even rates calculated in this manner cannot be considered as "absolute," but would depend upon criteria employed for the diagnosis of diabetes. Since our corresponding denominators were based only upon diabetics discovered as a result of having screened positive to any one of the five tests employed, we are using the terms "relative sensitivity" and "relative specificity" to connote this. We consider that within the limitations described certain useful information has been acquired.

MATERIALS AND METHODS

Persons in this study consisted of 3,309 applicants for employment health cards who applied for examination at the City of Memphis Hospitals outpatient department. These persons were referred by employers, employment agencies, or welfare agencies. Approximately 73 per cent were nonwhite, and 87 per cent of the latter were females. Nonwhite females therefore constituted the largest proportion of the group. Approximately 70 per cent of the total group were between the ages of thirty and fifty years. All but nine of the persons included in the study were over thirty years of age. Additional tests for syphilis, tuberculosis, uterine cancer, and glaucoma were also performed upon these persons.

Finger-tip blood (0.1 cc.) for the Clinitron test was drawn from all persons applying for a health card. The method of operation of the Clinitron and the Wilkerson-Heftman method of blood sugar determination which it employs have been fully described in the literature[1] and will not be detailed here. Suffice to say that the solution in which the blood and reagent tablets are placed remains blue when the preselected level (130 mg. in this study) is not exceeded, and turns colorless when this level is exceeded. The result is therefore read as "positive" or "negative." Reagent tablets required for the Clinitron are available commercially* and the cost of the four tablets required for each test is approximately five cents.

A specimen of urine was also obtained from each person examined. This was tested using Benedict's qualitative test, Clinitest, Tes-Tape, and Clinistix. The first two of these measure total reducing substances in urine, while the latter are enzyme tests which are specific for glucose. "Trace" results with Benedict's solution were ignored. This test was considered negative unless yellow, orange, or red precipitate was seen distinctly at the bottom of the tube after cooling and settling (about five minutes after the end of a five-minute period in the boiling water bath). Clinitest readings were made during the process of boiling as well as fifteen seconds after boiling ceased, in accordance with manufacturer's instructions. Results recorded with Tes-Tape and Clinistix also were in accord with the manufacturers' instructions.

Persons showing a positive test with any of the above blood or urine tests were recalled for a modified glucose tolerance test, in which the fasting blood sugar level and the level two hours after the administration of 100 gm. of glucose were determined. The Folin-Wu method was employed since "true" glucose determinations were not available from our hospital laboratory. Persons showing over 140 mg. on the two-hour blood sugar specimen of the modified glucose tolerance test were referred to the Medicine Clinic for definitive evaluation. However, for the purpose of this study, a diagnosis of diabetes was arbitrarily made, as recommended by Moyer and Womack,[7] when the two-hour specimen exceeded 140 mg. on the modified glucose tolerance test. Definitive evaluation, employing criteria recommended by the American Diabetes Association,[8] confirmed this arbitrary decision in all except four cases whose one-hour blood sugar level was not high enough to satisfy these criteria. The fasting blood sugar level was not considered in our arbitrary evaluation procedure but was useful as a check upon other laboratory reports.

ANALYSIS OF RESULTS

Table 1 indicates the distribution of new cases of diabetes found by all of the tests, by age group. Ninety cases of diabetes (2.7 per cent) were found in the 3,309 persons tested. The highest yield (10.4 per cent) were observed in the sixty- to sixty-nine-year age group.

The percentage with newly discovered diabetes found by each test is indicated in table 2. The percentage

* Eli Lilly and Company.

TABLE 1

Distribution of new cases of diabetes by age

Age group	Number tested	New diabetes Number	New diabetes Per cent
Under 30	9	0	0
30-39	1,227	14	1.1
40-49	1,084	26	2.4
50-59	678	24	3.5
60-69	212	22	10.4
70 and over	99	4	4.0
	3,309	90	2.7

found by the Clinitron blood test (2.4 per cent) was twice as high as the highest percentage found by any urine test (1.2 per cent for both Tes-Tape and Clinistix). In this same table, false positive tests are indicated both in relation to the total number of persons tested and to the number who screened positive by each test. Thus, 2.8 per cent of persons tested with the Clinitron blood test screened positive but turned out to be nondiabetic. This represents 52.9 per cent of those screening positive by this method and gives some measure of the retest load which failed to yield new cases of diabetes. Thus, slightly over one half of those screening positive with this test were found to be nondiabetic.

Benedict's test did not achieve a significantly lower retest load (45.5 per cent) of nondiabetics, even though it discovered less than one half as many diabetics (1.1 per cent) as did the Clinitron blood test (2.4 per cent).

Both enzyme tests for urine glucose achieved identical results in per cent new diabetics (1.2 per cent) and per cent false positive of those screening positive (39.4 per cent). Thus, these two tests discovered only half as many new diabetics as did the Clinitron, while the difference in retest load between these tests (39.4 per cent) and that of the Clinitron (52.9 per cent) was not of practical importance in the operation of our program.

TABLE 2

New diabetes and false positive tests in 3,309 persons

	Positive tests	New diabetes Number	New diabetes Per cent	False Positive Number	False Positive Per 100 tested	False Positive Per 100 positive
Clinitron	172	81	2.4	91	2.8	52.9
Benedict's	66	36	1.1	30	0.9	45.5
Tes-Tape	66	40	1.2	26	0.8	39.4
Clinistix	66	40	1.2	26	0.8	39.4
Clinitest	50	34	1.0	16	0.5	32.0

While the lowest discovery percentage of new diabetics of any of the tests was achieved by the Clinitest (1.0 per cent) the retest load (32 per cent) was substantially lower than that for the Clinitron (52.9 per cent).

The term "retest load" as employed above as a measure of specificity refers to the percentage of persons screening positive who were subsequently designated as nondiabetic. Strictly speaking those subsequently designated as diabetic were also subjected to the retesting process and should be included in the "retest load," but the former group appears to us to serve as a more realistic measure of the specificity or lack of specificity of a test.

Table 3 summarizes the relative sensitivity and relative specificity of the tests employed using as a standard of reference the number of new diabetics found by all tests. As noted in the previous data, the Clinitron blood test gave the best performance in relative sensitivity, and the Clinitest urine test, while performing at the lowest relative sensitivity level, gave the highest relative specificity.

TABLE 3

Relative sensitivity and relative specificity percentages for five tests in finding ninety diabetics in 3,309 persons

	Relative Sensitivity per cent	Relative Specificity per cent
Clinitron	90.0	97.2
Benedict's	40.0	99.1
Tes-Tape	44.4	99.2
Clinistix	44.4	99.2
Clinitest	37.8	99.5

Data presented up to this point have been based upon tests performed at random times in relation to eating. Table 4 combines information already presented with reference to per cent of new diabetes and per cent of false positives for each test, with data upon the time the test was performed since last food was eaten. It will be noted that for all of the tests the highest yield of new diabetes occurred when testing was performed between one and two hours after eating. Also, blood specimens examined by the Clinitron method revealed a higher percentage of new diabetes at all time periods in relation to eating than did the other tests. This superiority was most apparent on specimens taken during the period within one hour after eating (3.3 per cent versus 0.9 per cent for the others). The lowest yields of new cases were generally obtained

when testing was done on specimens taken under fasting conditions (including three hours after eating) or less than one hour after eating. The only exception to this was that the per cent of new diabetes found by the Clinitron blood test upon specimens taken within one hour after eating (3.3 per cent) was almost as high as that found between one and two hours after eating (4.0 per cent). Even under fasting conditions the per cent new diabetes found with the Clinitron blood test was as high (1.7 per cent) as the best performance of any of the other tests at any period in relation to eating. The consistently superior performance of the Clinitron blood test in recognizing new diabetes is therefore apparent.

The higher per cent of false positive tests obtained with the Clinitron blood test has already been noted, and is also evident in table 4 at all hourly time periods in relation to eating. However, the difference indicated between the per cent false positive of those screening positive with the Clinitron blood test and similar figures for the other tests was not considered to be of practical importance in our program except possibly for the difference observed between the Clinitron blood test and Clinitest urine test on fasting specimens. Clinitest showed the lowest per cent false positive tests (18.8 per cent of those positive) upon fasting specimens while still comparing favorably with the other tests with respect to the per cent of new diabetes found (0.9 per cent). Under many circumstances the Clinitest urine test would be the test of choice upon fasting specimens, especially where retest load is an important consideration.

DISCUSSION

Among the 3,309 persons in our study, 2.7 per cent were found to have previously unrecognized diabetes. Obviously one cannot generalize from a rate such as this regarding similar rates which might be encountered in other groups, whose characteristics may be different in important respects. The importance of adjusting rates for such factors as age is evident from our observation that persons in the sixty- to sixty-nine-year age group of our study showed a new diabetes rate almost ten times as high as that observed in the thirty- to thirty-nine-year age group. Also, the fact that 73 per cent of our study group were nonwhite and that 87 per cent of the latter group were females undoubtedly influenced our detection rate for new diabetes, since national mortality figures indicate a generally higher mortality rate for diabetes in nonwhites than in whites, and in nonwhite females than in nonwhite males. Another factor which may contribute to the detection rate of unrecognized diabetes in a group is the frequency with which its members undergo general physical examinations, either periodic or during episodes of illness, which may uncover subclinical diabetes. In a mathematical sense, the yield of previously unrecognized diabetes in any group from a detection program is therefore a function of many variables.

We have employed "relative sensitivity" and "relative specificity" rates in comparing the five diabetes detection tests of our study. Such rates have certain limitations, since they are based upon the total number of cases of new diabetes discovered by the simultaneous use of these tests, rather than being based upon the number discovered had it been possible to perform definitive diagnostic tests for diabetes upon all persons tested regardless of the result of the screening test. Nevertheless, these rates have provided us with useful information regarding the relative ability of the five tests under consideration to identify new cases of diabetes, and regarding the relative retesting load required

TABLE 4

Per cent new diabetes and per cent false positives in relation to last meal

	Time since eating							
	Fasting		Under 1 hour		1-2 hours		2-3 hours	
	Per cent new diabetes	Per cent* false positive	Per cent new diabetes	Per cent* false positive	Per cent new diabetes	Per cent* false positive	Per cent new diabetes	Per cent* false positive
Clinitron	1.7	53.7	3.3	57.7	4.0	44.0	2.1	59.5
Benedict's	0.9	23.5	0.9	75.0	1.4	44.4	1.3	47.4
Tes-Tape	0.9	33.3	0.9	50.0	1.7	33.3	1.4	47.6
Clinistix	0.9	30.0	0.9	50.0	1.7	36.8	1.4	47.6
Clinitest	0.9	18.8	0.9	57.1	1.3	25.0	1.1	40.0

*of those screening positive.

to achieve the performance observed for each test.

We have chosen to use as a measure of the retest load the per cent of persons screening positive by the test who were ultimately designated as nondiabetic upon further evaluation by other procedures. This has been considered by us as the "per cent false positive of those screening positive" category and is dependent in part upon the prevalence of diabetes in the population tested. We consider the size of this group to be of critical importance in any detection program for several reasons. The anxiety, as well as the expense incurred by a positive screening test does not generally appear to be compensated for by relief that the disease suspected is actually not present. Even when retesting is performed as part of the screening process at no expense to the patient, a negative final report is often received with mixed feelings. This is a problem inherent in all detection programs, since false positive screening results are an inevitable consequence of efforts to secure a maximum of true positive determinations.

Our comparison of blood and urine tests in diabetes detection revealed that the Clinitron blood test, when employed at the 130 mg. level, showed the highest relative sensitivity (90.0) of the tests under consideration. This rate was over twice as high as that of any of the other tests. While the retest load of false positive tests (52.9 per cent of those screening positive) was somewhat higher for this test than for the other tests, this difference is not of practical importance in our program excepting possibly in comparison to the Clinitest. There can be little question that the Clinitron blood test proved superior to the other tests employed, by virtue of greater relative sensitivity, and a relative specificity that was not markedly inferior to that of the other tests, except for Clinitest. The performance of the Clinitest in this respect is of interest, and under conditions where considerations of retest load may supersede those of enhanced sensitivity, the Clinitest may serve as a very acceptable detection test.

When the performance of the tests under study was examined in relation to time when food was eaten, it was of interest to note that even under fasting conditions the per cent new diabetes found with the Clinitron blood test was as high (1.7 per cent) as the best performance of any of the other tests at any period in relation to eating. The latter includes the period one to two hours after eating, when all tests achieved their best performance in this respect. At all of the hourly time periods in relation to eating, the Clinitron blood test was superior to the other tests in the discovery of new cases of diabetes. Concomitantly, the per cent false positive with the Clinitron blood test was also higher at each of these periods, although the differences observed are not of practical importance in our program except possibly in comparison with the Clinitest urine test on fasting specimens.

It is evident that under the conditions of our study, a blood testing procedure for determining whether or not a level of 130 mg. of glucose was exceeded proved to be the procedure of choice in the detection of previously unrecognized diabetes. Under circumstances where a low retest load upon fasting specimens is desirable, the Clinitest urine test would appear to be the test of choice.

It should be pointed out that in this study all tests were compared at maximum or close to maximum sensitivity. Different results would have been obtained had a level of 180 mg. been employed with the Clinitron.

SUMMARY

1. A comparison was made of the relative sensitivity and specificity of the Clinitron ("true" glucose) blood test, using the 130 mg. level, with four urine tests, Benedict's qualitative test, Tes-Tape, Clinistix, and Clinitest.

2. The Clinitron blood test showed the highest relative sensitivity, uncovering over twice as many new cases of diabetes as any of the urine tests.

3. While the retest load of false positive tests with the Clinitron blood test (53 per cent of those screening positive) was somewhat higher than for the urine tests, this difference was not considered to be of practical importance in our program, except possibly in comparison to the performance of Clinitest.

4. Even under fasting conditions, which are recognized as far from ideal for diabetes detection procedures, the per cent new diabetes found by the Clinitron blood test was equal to the best performance of any of the urine tests at any period in relation to eating.

5. Under the conditions of our study, blood testing at the 130 mg. level proved to be the diabetes detection method of choice when testing is done at random times in relation to eating.

6. Under circumstances where a low retest load upon fasting specimens is desired, the Clinitest urine test appears to be the test of choice.

SUMMARIO IN INTERLINGUA

Un Comparation de Tests de Urina e de Sanguine in le Detection de Diabete

1. Esseva interprendite un studio pro comparar le sensibilitate e le specificitate relative del test sanguinee a Clinitron ("ver" glucosa) al nivello de 130 mg con

illos de quatro tests urinari, i.e., le test qualitative de Benedict, le test a Tes-Tape, le test a Clinistix, e le test a Clinitest.

2. Le test sanguinee a Clinitron monstrava le plus alte sensibilitate relative. Illo detegeva plus que duo vices le numero de nove casos de diabete detegite per ulle del quatro tests de urina.

3. Durante que le requirimento de retestage in consequentia de false positivitate esseva plus alte in le caso del test sanguinee a Clinitron que in le tests de urina (53 pro cento del positivitates initial a Clinitron esseva false), iste differentia non esseva reguardate como un factor de importantia practic in nostre programma, excepte possibilemente in comparation con le comportamento de Clinitest.

4. Mesmo sub conditiones de jejunation (que recognoscitemente non representa un base ideal pro manovras de detection in diabete), le procentage del nove casos de diabete detegite per le test sanguinee a Clinitron esseva equal al melior resultatos obtenite per ulle del quatro tests de urina a non importa qual periodo de tempore in relation al ingestion de alimentos.

5. Sub le conditiones de nostre studio, le test sanguinee al nivello de 130 mg esseva le methodo de election in le detection de diabete quando le momento del test es sin fixe relation temporal al ingestion de alimentos.

6. In situationes in que un basse requirimento de retestage in specimens jejun es desirabile, le test de urina a Clinitest pare esser le test de election.

ACKNOWLEDGMENT

Acknowledgment is expressed to Mr. Robert M. Thorner, of the Chronic Disease Program, and Miss Nina V. Fisher, of the Atlanta Regional Office, United States Public Health Service, Department of Health, Education, and Welfare, for their assistance in the analysis of statistical data.

This project was aided in part by grants received from the Chronic Disease Program and from the Shelby County Tuberculosis and Health Association.

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On Teaching Diabetes

Correlation Clinics

Correlation clinics can be effective aids to medical teaching, but all too frequently these are held without adequate preparation. The clinician may spend two thirds of the hour presenting detailed clinical observations that mean little to the freshman medical student. The basic scientist follows, often simply repeating material that he has presented to the students previously. A correlation clinic given for freshmen in medical school should not be a predigested, capsule version of clinical medicine, even though the medical students are eager to hear a case history and to learn about treatment. They will have plenty of opportunity to do this in later years. Rather a correlation clinic should emphasize the way in which a given patient illustrates a particular aspect of anatomy, biochemistry, physiology, or endocrinology. I believe that to do a really adequate job the person preparing a correlation clinic should spend many hours in preparation, as much as ten to twenty hours for each correlation clinic. The participating clinician should know exactly what is being taught in the basic science departments, and he should seek out clinical material that will emphasize and illuminate the subject matter of the week. All too frequently, however, the most recently appointed instructor or even an intern or resident is asked to select the material, half an hour before the conference begins, and as a consequence the resulting clinic provides little correlation.

By Arnold Lazarow, M.D., in
Teaching and Research in Diabetes,
Charles C Thomas, Springfield,
Illinois, p. 47, 1960.

The Prevalence of Diabetes Mellitus in Jamaica

J. A. Tulloch, M.D., Jamaica, W. I.

Jamaica is the largest of the islands in the Federation of the West Indies, having an area of 4,411 square miles. It is situated at 18° north of the equator. The population of approximately 1,600,000 is predominantly Negro in origin.

Surveys of the prevalence of diabetes among nonwhite persons in four areas of Jamaica were carried out during 1959—60 with the assistance of the Government Medical Service. The areas chosen, Falmouth, Black River, Newport and Christiana, had a stable population which had recently been subjected to a house census by the Public Health Authorities. Falmouth and Black River are coastal towns on the north and south coast of Jamaica. Neither is particularly prosperous. Newport and the surrounding distict are representative of a rural area. Christiana is in the center of the island and the section surveyed was mainly urban but partly rural. The cooperation of the entire population aged fifteen years and over was sought and was obtained in Newport and almost so in Christiana. In the two fishing villages there was about 75 per cent cooperation. Each person was asked to provide a sample of urine passed two to three hours after the main meal of the day—generally the evening meal—and next day this was tested for sugar, using Tes-Tape. All showing glycosuria were then further investigated, the venous blood sugar being estimated in the fasting state and one hour after giving 50 gm. of glucose by mouth. All blood sugar estimations were done by the modified method of Nelson. A fasting sugar level of over 130 mg. per 100 ml. and a rise at one hour to 170 mg. or greater was considered to be diagnostic of diabetes.

RESULTS

The results, together with the findings of a pilot survey carried out in a rural area (Lawrence Tavern) in 1957,[5] are shown in tables 1 and 2. We studied 1,915 males and 2,601 females. Thirty males and fifty-eight females had glycosuria. Fourteen (four males and ten females) could not be investigated further and in seventeen (eight males and nine females) diabetes was excluded on the blood sugar results. There remained fifty-seven proved diabetics—eighteen males and thirty-nine females, giving an incidence of 0.94 per cent for males and 1.5 per cent for females with a combined figure of 1.26 per cent. Twenty-five of the diabetics were known to have diabetes prior to the survey and thirty-two were newly discovered.

From the University College of the West Indies, Jamaica, W. I.

DISCUSSION

The figures suggest that there is a higher incidence of diabetes in the urban areas—Black River, Falmouth and Christiana—than in the rural districts, Newport and Lawrence Tavern. They also reveal the greater frequency of diabetes among the middle-aged females in the community. The prevalence rate of 1.26 per cent is low, however, when compared with the findings in Britain (Ibstock, Walker, 1959) and in the United States (Oxford, Wilkerson and Krall, 1947) when comparable age groups (i.e., fifteen years and over) are considered —1.7 per cent in Britain and 2.7 per cent in the United States (table 3). This is probably partly due to the age distribution of the population surveyed—in Jamaica 33 per cent of the males and 30 per cent of the females were forty-five years of age or older, whereas in Britain 45 per cent of the males and 46 per cent of the females and in the United States 37.6 per cent of the males and 37.2 per cent of the females were in this age group (table 3). The findings were also lower than those reported from Trinidad[8] where it was found that 1.4 per cent of 2,325 employes (aged fifteen years and over, African and East Indian) of Shell Trinidad Ltd. had diabetes. This figure is all the more surprising in that only 15.5 per cent of the males and 11.1 per cent

TABLE 1

Occurrence of diabetes in five surveys carried out in Jamaica

	Male	Female
Total subjects investigated	1,915	2,601
Total with glycosuria	30	58
Diabetes excluded	8	9
Not investigated further	4	10
Diabetic	18	39
Per cent incidence of diabetes	0.94	1.5

TABLE 2

Age and sex distribution of the population studied in five diabetic surveys carried out in Jamaica. The Lawrence Tavern results have been previously reported (Tulloch & Johnson—1958)

Age in years	Newport				Christiana				Black River				Falmouth				Lawrence Tavern				Total			
	Male		Female		Male		Female		Male		Female		Male		Female		Male		Female		Male		Female	
	Total	D	Total	D	Total	D	Total	D	Total	D	Total	D	Total	D	Total	D	Total	D	Total	D	Total	D	Total	D
15-19	52	0	86	0	46	0	83	0	39	0	59	0	45	0	86	0	51	0	74	0	233	0	382	0
20-29	78	0	118	0	124	0	143	0	92	0	136	0	95	0	162	0	78	0	116	0	467	0	675	0
30-39	75	0	95	1	66	1	111	1	68	0	75	0	88	0	132	0	72	1	101	0	369	2	514	2
40-49	91	0	96	1	98	2	102	2	54	1	57	0	67	1	108	3	82	1	110	1	392	5	473	7
50-59	53	0	43	1	59	2	79	3	33	1	35	1	49	0	73	3	69	1	66	3	263	4	296	11
60-69	22	0	44	0	29	1	30	3	15	3	15	4	21	0	38	9	40	1	37	0	127	5	162	16
70-79	7	0	13	0	9	0	13	0	7	1	8	1	13	0	27	2	12	0	10	0	48	1	71	3
80+	3	0	10	0	8	0	1	0	0	0	2	0	3	1	10	0	2	0	5	0	16	1	28	0
Total	381	0	505	3	439	6	562	9	308	6	387	6	381	2	630	17	406	4	519	4	1,915	18	2,601	39

D = Diabetic

TABLE 3

Details of recent diabetes surveys

Country	Population surveyed*			Number of diabetics	Percentage of incidence of diabetes	Percentage of population aged forty-five years and over	
	Male	Female	Total			Male	Female
Jamaica	1,915	2,601	4,516	57	1.26	33	30
Britain (Walker 1959)	1,636	1,637	3,273	56	1.7	45	46
United States of America (Wilkerson & Krall 1947)	1,226	1,359	2,585	70	2.7	37.6	37.2
Trinidad (Wright & Taylor 1958)	1,291	1,034	2,325	32	1.4	15.3	11.1
Southern Rhodesia (Carr & Gelfand 1960)	570	437	1,007	1	0.1	7.3§	6.2§
Basutoland (Politzer et al. 1960)†	673	2,327	3,000	7	0.23	52§	31.6§
Ghana (Dodu 1958)‡	2,408	760	3,168	16	0.5	7	12.1

*Excluding cases under fifteen years in all surveys. †This study is of hospital outpatients aged twenty years and over. ‡Hospital outpatient study. §Population aged forty years and over.

In the surveys listed above the diagnosis of diabetes was established by the following methods:

Walker—Glycosuria and glucose tolerance test.

Wilkerson & Krall—Glycosuria and postprandial blood sugar above 170 mg. per 100 ml. (venous) or 200 mg. per 100 ml. (capillary), with glucose tolerance test on borderline cases.

Wright & Taylor—Glycosuria and fasting and postprandial blood sugar with, if necessary, glucose tolerance test.

Carr & Gelfand—Details not given.

Politzer et al.—Postprandial glycosuria and blood sugar.

Dodu—Glycosuria and blood sugar and, if necessary, a glucose tolerance test.

of the females were forty-five years and over.

On the other hand, our figure of 1.26 per cent is very much greater than the 0.1 per cent reported by Carr and Gelfand (personal communication, 1960) from the African township of Highfield in Southern Rhodesia; the 0.23 per cent reported by Politzer et al.[4] among 3,000 adult hospital outpatients in Basutoland and the 0.5 per cent (excluding those under fifteen years) found in hospital outpatients in Accra, Ghana.[2] In both Highfield and Accra the proportion of the elderly population studied was very low—in Highfield, 7.3 per cent of the males and 6.2 per cent of the females were over forty years; in Accra, 7 per cent of the males and 12.1 per cent of the females were over forty-five years. In the Basutoland study only adults of twenty years and over were studied and 52 per cent of the males and 31.6 per cent of the females were over forty years. The wide range of figures quoted for the occurrence of diabetes are of great interest but the striking differences in the age distribution of the population in the different areas make strict comparison of the prevalence figures unjustifiable and it is also not advisable to compare survey findings with studies of hospital outpatients. However, by applying the diabetes rates per 100,000 population as found in the Jamaican surveys, to the populations surveyed in Britain, the United States and Trinidad, the

effect of the differing age distributions may be canceled. The number of diabetics which might therefore be expected in each population is shown in table 4 from which it is clear that diabetes is as common in Jamaica as in Britain, but that it is less common than in the United States and Trinidad.

TABLE 4

Age correction of diabetic survey findings to Jamaican diabetic survey rates

		Diabetics found in survey	Number expected
BRITAIN	Male	18	22
	Female	38	38
U.S.A.	Male	31	13
	Female	39	25
TRINIDAD	Male	19	8
	Female	13	6

The prevalence rate per 100,000 population for each decade of the population surveyed in Jamaica has been applied to the population of each decade in the British, American and Trinidad surveys to give the expected number of diabetics in Jamaica were the population distribution the same as in the other three areas.

If the figure of 1.26 per cent obtained by combining the five surveys in Jamaica is applied to the island as a whole there are about 12,000 diabetics in Jamaica at this time of whom only about 40 per cent are known to have the disease and are therefore available for treatment.

SUMMARY

The prevalence of diabetes in Jamaica has been investigated. The results of five surveys are reported. The indications are that 1.26 per cent of the population aged fifteen years and over have diabetes.

SUMMARIO IN INTERLINGUA

Le Prevalentia de Diabete Mellite in Jamaica

Le prevalentia de diabete mellite in Jamaica esseva investigate. Le resultatos de cinque enquetes es reportate. Le datos indica que 1,26 pro cento del population de etates de dece-cinque annos e plus ha diabete.

ACKNOWLEDGMENT

I am grateful to the following Government Medical Officers and their staff for the assistance given in carrying out the surveys: Dr. A. A. Peat, Chief Medical Officer, Jamaica; Dr. D. B. Bryson, Falmouth; Dr. A. Russell, Black River; and Dr. E. C. Dymond, Newport and Christiana. I also wish to thank Dr. F. B. Peck, Sr., of Eli Lilly and Company for supplying us with Tes-Tape.

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Linoleic Ester and Cholesterol

Several experiments have been performed recently to determine the nutritional effects of single unsaturated fatty acids. Such studies include the review by I. H. Page et al. (*Circulation* 16:163, 1957) and the experiments of J. T. Anderson, A. Keys, and F. Grande (*J. Nutrition* 62:421, 1957) in which diets of controlled fat content were fed to adult male human subjects. The fats studied included butterfat, olive oil, cottonseed oil, corn oil, sunflower seed oil, coconut oil, sardine oil, and "house diet" fat. They observed that the serum cholesterol response correlated roughly with the principle that saturated fat diets led to higher cholesterol levels than polyunsaturated fat diets, but that neither degree of unsaturation nor content of linoleic acid explained the results.

In the study reported by D. M. Hegsted, A. Gotsis and F. J. Stare (*J. Nutrition* 63:377, 1957) a large number of oils were tested in cholesterol-cholic acid-fed rats. They observed that "nonessential" unsaturated fatty acids promoted hypercholesteremia which was counteracted either by essential fatty acids or saturated fatty acids.

From *Nutrition Reviews*, Vol. 19, No. 4, pp. 117-18, April 1961.

Studies Concerning a Possible Humoral Factor Produced by Working Muscles

Its Influence on Glucose Utilization

W. E. Dulin, Ph.D., and J. J. Clark, D.V.M., Ph.D.,
with the technical assistance of G. H. Lund and H. Albert, Kalamazoo

It is generally accepted that glucose utilization, as measured by fall in blood sugar, is enhanced by exercise in intact, diabetic or eviscerate animals[1-4] as well as in diabetic humans.[5-6] This increased utilization is probably due to an increased oxidation since, in intact rats, exercise results in an increase in C-14O_2 when worked animals are infused with uniformly labeled glucose.[3]

A hypothesis that the influence of muscle work is mediated by a humoral factor has been suggested by Goldstein and Levine[2] and was based on two observations: (1) stimulation of one group of muscles led to distribution of galactose in total body water, and (2) denervation did not influence this distribution. This hypothesis has recently been extended by Goldstein[7] in studies showing that cross-circulation of blood from working to resting dogs caused a depression of blood sugar in the resting recipient animals. Opposed to the humoral hypothesis are the data of Helmreich and Cori[8] and Sacks and Smith,[25] which show that a resting gastrocnemius muscle did not exhibit an increased uptake of pentoses when the other musculature of the legs was stimulated.

Muscle work has been compared to insulin in several instances. These comparisons have led to the conclusion that some of the metabolic effects of insulin and exercise are similar while others are different. Both insulin and exercise are reported to increase glucose oxidation[3] and the same sugars are responsive to both insulin treatment and exercise.[2] This finding, however, has recently been challenged.[8] In contrast, insulin has been shown to reduce and exercise to increase plasma amino acids in eviscerate rats.[20] In addition, insulin causes a depression of ketone bodies and fat[21] while exercise can cause acidosis,[5] implying increased mobilization of fatty acids from depot fats. It seems likely therefore that if a humoral factor is elaborated by working muscles it does not mimic the metabolic effects of insulin.

The purpose of the studies described here has been two-fold: (1) to investigate some of the changes in carbohydrate metabolism during exercise and compare these effects with insulin, and (2) to examine the hypothesis that working muscles release a factor which influences glucose utilization by resting muscles. The technics involved the measurement of arterial-venous glucose differences across resting and worked legs of eviscerate dogs and accumulation of galactose C-1-14 in resting and worked muscles of eviscerate rats.

Presented at the Twenty-first Annual Meeting of the American Diabetes Association in New York City on June 24, 1961.

From the Research Division of The Upjohn Company, Kalamazoo, Michigan.

METHODS AND RESULTS

Influence of muscle work or insulin on oxidation of C-14 uniformly labeled glucose by eviscerate rats.

The influence of muscle work or insulin was determined on eviscerated, male, Upjohn rats (Sprague-Dawley ancestry), weighing 230 ± 5 gm. Evisceration was accomplished by the method of Russell[10] while the animals were under anesthesia induced by intraperitoneal injection of Cyclopal sodium* (30 mg./kg.) and subcutaneous injection of phenobarbital sodium (130 mg./kg.). The adrenals and kidneys were left intact. Immediately following the operation, the animals were connected to a constant infusion pump which delivered 80 mg. of unlabeled glucose per 100 gm. of body weight per hour in 1.25 cc. of saline. The C-14 uniformly labeled glucose† was infused at a count of 1.8 x 10^6 CPM/rat/hour for the muscle work experiments and 9 x 10^5 CPM in the insulin experiments. The glucose infusion was continued for fifteen minutes prior to initiation of work or the subcutaneous injection of insulin.

Expired CO_2 was collected by placing each animal in an individual chamber designed for this collection. A negative pressure air flow passed through each chamber

* 5-(1-cyclopenten-2-yl)-5 allylbarbituric acid sodium.

† Obtained from Volk Chemical Company, Chicago, Illinois.

and pulled the expired air into a sodium hydroxide trap. The air entering the chambers was filtered through Drierite and Ascarite. The sodium hydroxide was sampled at 30, 60 and 90 minutes after initiation of work or insulin injection. The CO_2 was precipitated as barium carbonate, plated and counted in a gas flow counter. Specific activity of the CO_2 is expressed as counts/minute/milligram of barium carbonate which was corrected for self-absorption.

The chambers housing the animals were wired so that muscle stimulation could be accomplished while the animals were inside. Stimulation at a rate of five per second with a duration of 20 msec. and an intensity of 20 ma. was carried out, using a nerve stimulator (Upjohn-Model B). The animals worked against a 100 gm. weight which was tied to the tendon of the left gastrocnemius. The stimulus was passed from the right back foot to the left back foot in order that the entire musculature of both legs was worked. The chambers housing the animals were placed in a constant temperature box maintained at 26.5 ± 0.5° C. The resting animals were handled like the worked rats except that the switch controlling the impulse from the stimulator remained open.

Within thirty minutes after initation of muscle work, the per cent of infused glucose oxidized was increased sevenfold (figure 1). At ninety minutes there was a tenfold increase in glucose oxidation by the working rats. Insulin did not produce any marked effect until sixty minutes, and at ninety minutes the insulin-treated animals oxidized only four times as much glucose as the controls (figure 2). The greater effect of exercise on glucose oxidation may be due to the timing or dose of the insulin, or to the larger dose of glucose U-C-14 used in the work study.

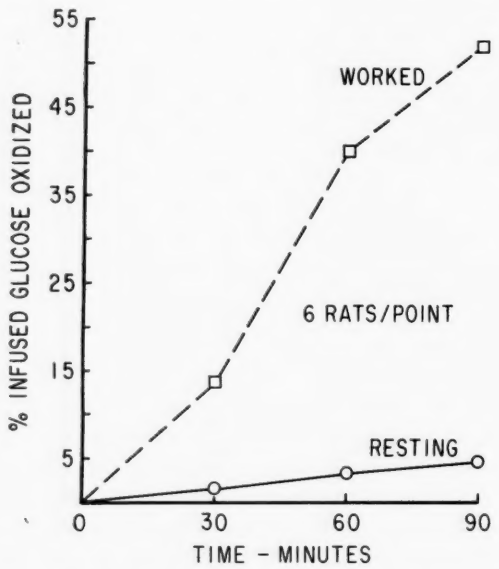

FIG. 1. Effects of muscle work on glucose oxidation in eviscerate rats. Unlabeled glucose at 80 mg./100 gm./hr., glucose-U-C-14 at 1.8 x 10^6 CPM per hour.

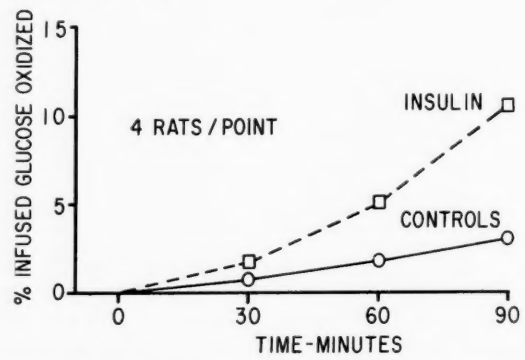

FIG. 2. Effects of subcutaneously administered insulin on oxidation of glucose in eviscerate rats. One unit of Crystalline Insulin/rat. Unlabeled glucose infused at 80 mg./100 gm./hr., glucose-U-C-14 at 9 x 10^5 CPM per hour.

The weight of the barium carbonate from the exercised animals was decidedly greater than that from their controls. Insulin had no marked effect on total barium carbonate (table 1). The specific activity of the barium carbonate (and indirectly of the CO_2) was increased following either muscle work or insulin treatment. These data were interpreted to mean that both insulin and muscle work caused a relatively specific increase in glucose oxidation.

Influence of muscle work or insulin on glycogen content of the diaphragm, gastrocnemius or triceps muscles.

Since one of the most reproducible effects of insulin is that of stimulating glycogen accumulation in the diaphragm, it was of importance to determine if muscle work was capable of duplicating this.

Male rats of the Sprague-Dawley strain weighing 225 ± 4 gm. were functionally eviscerated as previously described. They were placed on operating boards in the constant temperature box instead of individual airtight chambers and were infused via the jugular vein with a glucose load of 80 mg./100 gm. body weight/1.25 cc. saline/hour. Insulin (Crystalline Zn) when used was injected subcutaneously at a dose of 1.0 unit/rat. Blood and tissues were removed for chemical analyses ninety minutes following insulin injection or initiation of muscle work. Blood sugar was determined by the Nelson procedure[11] and glycogen by the anthrone method.[12]

TABLE 1

Effects of muscle work or insulin on specific activity of expired CO_2 (CPM/mg. barium carbonate) in eviscerated rats. Experiments were ninety minutes in duration. Unlabeled glucose infused at 80 mg./100 gm./hr., glucose-U-C-14 at 1.8×10^6 CPM/hr. in the work experiments and 0.9×10^6 CPM in the insulin experiment. CPM were corrected for self-absorption by a calibration curve for $BaCO_3$ for the instrument used (Baird-atomic gas flow).

Time	Controls (6) Weight $BaCO_3$ (mg.)	CPM	S.A.	Worked (6) Weight $BaCO_3$ (mg.)	CPM	S.A.
30	2,553	2.53×10^4	9.87	3,200	2.8×10^5	87.5
60	4,200	7.07×10^4	16.8	5,230	8.49×10^5	162.3
90	5,146	1.39×10^5	27.0	7,295	1.51×10^6	206.9
	Controls (4)			Insulin (4)		
30	2,765	1.13×10^4	4.1	2,390	1.9×10^4	7.94
60	5,695	3.7×10^4	6.5	4,181	9.3×10^4	22.3
90	5,925	8.4×10^4	14.2	6,076	2.5×10^5	41.4

Insulin, as anticipated, increased the diaphragm glycogen from 0.35 to 0.49 per cent (table 2). It was ineffective at this dose, however, in causing any change in the glycogen of either the triceps or gastrocnemius muscles. Muscle work did not alter diaphragm or triceps glycogen but the glycogen of the exercised muscle (gastrocnemius) was reduced from 0.37 to 0.13 per cent (table 2). Both insulin and muscle work produced similar decreases in the blood sugar.

TABLE 2

Comparative effects of insulin and muscle work on diaphragm, triceps and gastrocnemius muscle glycogen and blood sugars of eviscerated rats. Experiments ninety minutes in duration. Glucose infused at 80 mg./100 gm./hr. Insulin given subcutaneously

No. rats	Blood sugar	Treatment	Tissue glycogen—per cent wet weight: Diaphragm	Gastrocnemius	Triceps
9	277	Resting	.21	.37	.30
8	100	Exercised	.20	.13†	.27
6	309	—	.35	.47	.41
6	128	Insulin (1 unit*)	.49	.49	.38

* Crystalline Zn (Iletin).
† Represents exercised muscle.

Comparative effects of muscle work and insulin on the glycogen content of epididymal fat.

Muscle work was accomplished by the procedure described in the previous section in functionally eviscerated rats (Sprague-Dawley). The insulin was injected subcutaneously. The epididymal fat pads were removed ninety minutes following the insulin treatment or beginning of the work and digested in 2 cc. of hot 30 per cent potassium hydroxide. Following digestion, 3 cc. of 95 per cent ethyl alcohol was added and the mixture again brought to a boil. After standing overnight in the refrigerator, the digest was centrifuged and the supernatant discarded. The precipitate was washed twice with hot 95 per cent ethyl alcohol and the glycogen was determined quantitatively by the anthrone procedure.[12] Blood sugars were determined in duplicate by the Nelson method[11] at the end of the experiment on blood obtained from the vena cava.

The epididymal glycogen was increased from .0112 per cent to .0223 per cent by insulin treatment while the blood sugar was markedly depressed (table 3). Although muscle exercise produced an even greater fall in blood sugar than did insulin, it failed to cause any change in the glycogen content of the epididymal fat. These results show another important difference between the biological effects of insulin and exercise and suggest that, if exercise does produce a humoral factor, it is not insulin-like.

TABLE 3

Comparative effects of insulin and muscle work on glycogen content of epididymal fat of eviscerated rats. Experiments ninety minutes in duration

No. rats	Treatment	Dose	Epididymal fat glycogen (per cent wet weight)	Blood sugar
5	Control		.0112	191
6	Insulin*	1.0 unit	.0223	91
6	Resting		.0115	231
5	Exercised		.0108	83

* Crystalline Zn (Iletin).

Duration of effect of muscle work on blood sugar.

Male Upjohn rats (230 ± 5 gm.) were used in this study. The animals were functionally eviscerated and both hind legs were worked using the procedure de-

scribed previously. The animals were infused with a glucose load of 80 mg./100 gm. body weight/1.25 cc. saline/hour. Blood sugars were determined by the Nelson method[11] on tail blood sixty minutes after the beginning of work. Glucose infusion was continued for sixty minutes following cessation of work and sugars were again determined.

Results (table 4) show that the blood sugars of the worked rats were 51 mg. per 100 ml. as compared to 187 mg. per 100 ml. in the controls at the end of the 60-minute work period. During the hour after termination of work, the blood sugars of the controls increased by 131 mg. per 100 ml. as compared to a 49 mg. per 100 ml. increase in the previously worked rats. It can therefore be concluded that the effects of severe exercise on glucose utilization persist for at least one hour.

TABLE 4

Effect of muscle work on utilization of glucose after cessation of work. Animals worked for one hour, stimulation stopped and glucose infusion continued for one additional hour. Glucose at 80 mg./100 gm./hr.

No. rats	Treatment	Blood sugars 1 hr.— immediately after stopping	Blood sugars 2 hr.—1 hr. after stopping	Change 1—2 hr. mg. per 100 ml.
6	Resting	187	318	+131
5	Worked	51	100	+ 49

Effects of muscle work on arterial-venous (A-V) glucose across worked and resting legs of eviscerate dogs.

Healthy, intact, mongrel female dogs weighing 12-18 kg. were used in these studies following an overnight fast. Evisceration was carried out by a one-stage technic[13] while the animals were under Nembutal* anesthesia. All animals were functionally nephrectomized by ligation of the arterial and venous blood supply of the kidneys.

The resting animals received an intravenous dose of 2 gm. of glucose immediately following the surgery and were then connected to a constant infusion pump which delivered glucose at a rate of 133 mg./kg. in 12.5 cc. saline/hour. The worked dogs received 3 gm. glucose immediately following the operation and a glucose load of 200 mg./kg./12.5 cc. saline/hour. It was found by preliminary experiments that this dose of glucose was required to keep the blood sugars of the working animals at the same level as those of the controls. Work was accomplished by stimulation of both hind legs directly by intramuscular electrodes connected to a nerve stimulator (Upjohn-Model B). The rate of stimulation was 3/sec. against a 2 kg. weight at an intensity of 30 ma and a duration of 20 msec. Body temperatures were maintained at 95.5 to 96.5° F. by electric heating pads.

Glucose infusion was started thirty minutes prior to a 0-hour blood sample taken from the jugular vein and muscle work was initiated immediately thereafter. Blood samples were also taken from the jugular at 30, 60 and 90 minutes. At the end of ninety minutes, work was stopped and the brachial and femoral veins, as well as the dorsal aorta, were exposed, cannulated and the cannulae filled with a weak heparin solution. This procedure required approximately fifteen minutes. Simultaneous blood samples were taken from these vessels at 0, 15, 30, 45 and 60 min. following completion of cannulation. The fact that the effects of muscle work persist for an hour after stopping work supports the usefulness of determining A-V glucose differences after cessation of exercise.

It is apparent that the blood sugar of the worked and resting dogs increased to the same extent even though the worked animals received a 50 per cent greater glucose load (figure 3). Therefore, exercise under the conditions used actually increased glucose utilization as measured by blood sugar levels. Examination of the data on A-V glucose difference in resting animals shows that the front (aorta-brachial) and hind (aorta-femoral) legs were essentially the same with an average A-V difference of approximately 14 mg. per cent. The A-V glucose differences of the resting legs of the exercised dogs (average 14.5 mg. per cent were similar to those of the resting animals (14 mg. per cent). In marked contrast, the A-V differences were increased to an average of 36 mg. per cent across the worked leg (aorta-femoral) of the exercised animals. The A-V glucose differences across the exercised and resting legs of the worked animals are significantly different (P=.01 or less) at each time interval. The standard errors of the means (shaded area) also indicate that these differences are real.

It is therefore concluded from these data that glucose utilization is increased only in the working muscles. These observations do not support the hypothesis that worked muscles release a humoral agent which can cause increased glucose utilization by distal resting muscles.

Effects of muscle work on galactose-1-C-14 accumulation, raffinose space and total tissue water of eviscerate rats.

The influence of muscle work on the transfer of sugars into working and resting muscles of functionally eviscerated rats was studied by measuring intracellular concentration of galactose-1-C-14 in the muscle. Galactose was used since it was found by preliminary experiments that less than 1 per cent of an infused dose was oxidized

* 5-Ethyl-5-(1-methylbutyl) barbituric acid.

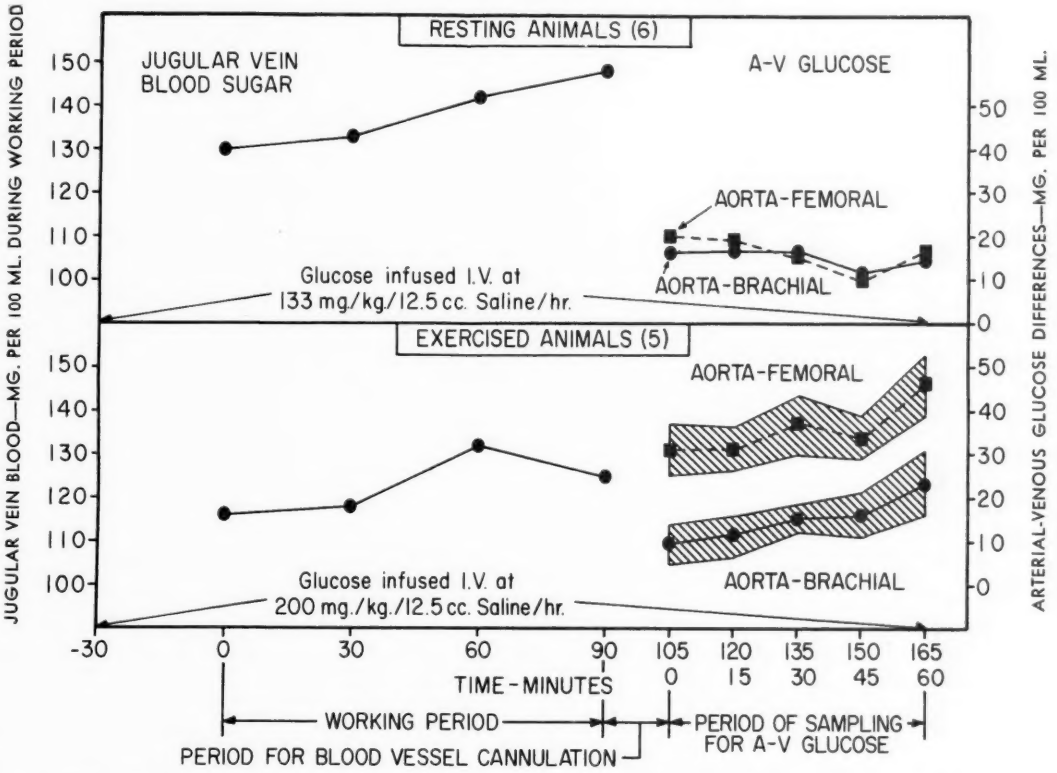

FIG. 3. Effect of exercise on arterial-venous glucose across worked and nonworked limbs of eviscerated dogs.

to CO_2 and that the oxidation rate was not significantly changed by either exercise or insulin in the eviscerate rats. Since galactose distribution is increased with insulin[2] and since it is essentially nonmetabolizable by muscle, it can be used for studies of this nature.

The evisceration and muscle work procedure was carried out as described above. The rats used in these studies were obtained from Charles River (Sprague-Dawley) and weighed 225 ± 5 gm. Glucose was infused at 80 mg./100 gm. body weight/hour, together with galactose-1-C-14 at a concentration calculated to result in an infusion rate of 3 x 10^6 CPM/rat/hour. The CPM of the infusion solution was determined by counting in a Packard Tri-carb Model 314 DC liquid scintillation spectrometer. The experiments were of ninety minutes' duration. Immediately after the gastrocnemius and triceps muscles were removed, a terminal blood sample was taken (3 ml.). The muscles were homogenized in 20 ml. of 5 per cent trichloroacetic acid (TCA) with a Servall tissue homogenizer, and then diluted to 50 ml. Blood was processed by adding 0.5 ml. to 9.5 ml. of 5 per cent TCA. The blood and tissue extracts were then centrifuged and samples of the supernatant were taken for counting.

The counting was done by adding 0.5 ml. supernatant of tissue extracts to 20 ml. of counting fluid. The counting fluid was made with 700 ml. triple distilled toluene, 300 ml. absolute ethanol, 3 gm. diphenyl-oxazole, and 100 mg. of 1,4-di(2-(5-phenyl-oxazolyl) benzene). Counting was done in a Packard Tri-carb Model 314 DC liquid scintillation spectrometer. Counts are determined as counts per minute per sample without correcting for efficiency of the instrument. All samples were checked for quenching, which was negligible in all cases.

Raffinose space was also determined, although because of technical difficulties, on different groups of rats than used for the galactose-1-C-14 studies. The usefulness of the raffinose method for determining extracellular space has been discussed in detail by others.[8,9] In these experiments, the animals were worked for ninety minutes and then 100 mg. of raffinose/100 gm. body weight was infused intravenously. Muscles and blood were obtained

thirty minutes following the infusion. The raffinose was determined chemically by the method of Roe et al.[15] Extracellular space was determined by the formula: CM/CP x 100, where CM is the concentration of raffinose in 100 cc. of muscle water, and CP is the concentration in 100 cc. of plasma water. The total muscle, blood, and plasma water were determined by drying to a constant weight in a vacuum oven at 100°C. The intracellular concentration of galactose-1-C-14 was calculated by the expression:

$$Ci = \frac{C_t - (C_b \cdot E)}{(1.0 - E)}$$

where Ci is intracellular CPM (counts per minute), Ct is CPM in the total muscle water, C_b is CPM in the blood, and E is the raffinose space.

Total muscle, blood, and plasma water were not significantly changed by muscle work. The plasma water was 92.8 per cent in the working animals as compared to 93.3 per cent in the resting rats. The total water content was identical in the front and hind leg muscles of the resting rats. In the exercised rats, the total water of the worked muscles (gastrocnemius) was slightly greater (1.1 per cent) than that of the resting muscles (triceps) (table 5).

TABLE 5

Effects of exercise on total tissue water of plasma, blood, triceps and gastrocnemius muscles. Determined on tissues obtained ninety minutes after beginning muscle work. Water determined by drying to constant weight in a vacuum oven at 100° C.

Tissue	Per cent water	
	Resting animals (6)	Worked Animals (5)
Plasma	93.3	92.8
Blood	79.9	79.8
Triceps	76.4	76.6
Gastrocnemius	76.3	77.7*
Blood sugar	278	59

* Represents worked muscle.

Extracellular space was found to be the same for worked or resting muscles. There was a slight difference in raffinose space between the triceps (25 per cent) and gastrocnemius (26.8 per cent) of the resting animals. However, these differences are not significant and fall within the limits of error of the method used (table 6).

Intracellular galactose-1-C-14 was not demonstrable in the muscles of the resting animal (table 7) nor the triceps (resting) of the exercised animal. There was a marked accumulation of galactose-1-C-14 in the worked muscles, however (table 7). These results are interpreted to mean that the influence of work is essentially limited to the working muscles.

TABLE 6

Extracellular space (ES) of muscles of worked and resting rats. Determined by raffinose determinations using the formula: $ES = \frac{\text{mg. Raffinose/100 ml. Muscle Water}}{\text{mg. Raffinose/100 ml. Plasma Water}} \times 100.$

Animals worked for ninety minutes and raffinose injection I.V. at 100 mg./100 gm. body weight. Tissues removed thirty minutes following raffinose infusion.

Tissue	Resting rats (11)		Exercised rats (11)	
	Raffinose conc. per 100 ml. water	Extracellular space (per cent)	Raffinose conc. per 100 ml. water	Extracellular space (per cent)
Plasma	1.84		1.78	
Triceps	0.376	25	0.404	27.4
Gastrocnemius	0.402	26.8	0.409*	27.4*
Blood sugar	267		41	

* Represents worked muscle.

Effects of muscle work on body temperature and the influence of temperature changes on glucose disposal in eviscerated rats.

During experiments designed to study the influence of exercise on glucose utilization, it was observed that worked rats were hyperthermic. Since the possibility existed[16,17] that increased body temperature could increase glucose disposal, experiments were designed to determine the role of body temperature in lowering blood sugar of eviscerate rats.

Sprague-Dawley male rats (230 ± 5 gm.) were eviscerated and exercised by our standard method. Temperatures were measured by a Tri-R electric thermometer. Table 8 summarizes data showing that two hours of exercise at a temperature of 26.5 ± 0.5° C. causes an increase in body temperature of approximately 10° F. To determine if this temperature change was responsible for changes in blood sugar, rats were put in chambers maintained at 26.5° C., 35° C., and 37.5° C., and kept in this environment for two hours. Blood sugars were determined after two hours by the Nelson method.[11] It can be seen that both the 35° and 37.5° C. environments caused an increase in body temperature similar to that produced by muscle work (table 9). It is apparent, however, that this increased temperature did not cause a reduction of blood sugar. It must be concluded that increased body temperature is not the cause of increased glucose oxidation but is secondary.

TABLE 7

Effects of exercise on intracellular concentration of galactose-1-C-14 in resting and worked muscles. Work carried out for ninety minutes. Galactose infusion at 3×10^6 CPM/rat/hour. Glucose load of 80 mg./100 gm./hour. Intracellular concentration determined as CPM/ml. water by the formula: $Ci = \frac{C_t - (C_b \cdot E)}{(1-E)}$ where Ci = intracellular concentration (CPM), E = extracellular space, C_t = concentration in total muscle water (CPM), and C_b = concentration in blood (CPM).

	Galactose concentration			
	Resting animals (6)		Exercised animals (6)	
Tissue	Conc./ml. Tissue water (CPM)	Intracellular conc./ml. (CPM)	Conc./ml. tissue water (CPM)	Intracellular conc./ml. (CPM)
Blood	4.4×10^4		3.2×10^4	
Triceps	1.2×10^4	0	8.2×10^3	0
Gastrocnemius	1.15×10^4	0	2.8×10^4	2.64×10^4
Blood sugar		211		36

TABLE 8

Effects of muscle work on body temperature and blood sugar of eviscerated rats in a 26.5° C. environment. Worked ninety minutes. Glucose load of 80 mg./100 gm. body weight/hour.

		Body temperature		
No. rats	Treatment	Before work	After work	Blood sugar (mg. per 100 ml.)
		Experiment I		
7	Resting	88° F.	86.5° F.	
8	Worked	89° F.	95.5° F.	
		Experiment II		
5	Resting	88° F.	87° F.	324
5	Worked	87° F.	97° F.	87

TABLE 9

Effects of environmental temperature on body temperature and blood sugar of eviscerated rats. Animals kept at temperature for two hours. Glucose load of 80 mg./100 gm./hr.

		Body temperature		
No. rats	Conditions	Before	After	Blood sugar
		Experiment I		
5	26.5° C.	87° F.	87° F.	171
5	37.5° C.	86° F.	99° F.	211
		Experiment II		
5	26.5° C.	88° F.	85° F.	260
5	35.0° C.	89° F.	99° F.	293

DISCUSSION

These studies were based on the hypothesis that exercising muscles elaborate a humoral factor which is capable of causing an increased glucose utilization by resting muscles. The study on the influence of work in the oxidation of glucose-U-C-14 to $C\text{-}14O_2$ in eviscerate animals was considered basic since the entire premise of a humoral muscle work factor assumes utilization of glucose by oxidation rather than storage or disposal by some other means. The data showing that $C\text{-}14O_2$ from infused glucose-U-C-14 is increased in eviscerate rats subjected to work or treated with insulin supports the conclusion that the glucose disappears, at least in part, as a result of complete oxidation. These results confirm and extend those of Ingle et al.[3] who found that exercise increases glucose oxidation by intact rats. It was also noteworthy that the effect of exercise was greater than that of insulin in these experiments. Whether the lesser effect of insulin was due to a true quantitative difference or to nonoptimal insulin time or dosage cannot be determined from these data, however. These results showing exercise to have a greater effect than insulin are in agreement with those of Kipnis et al.[9]

Certain observations reported here strengthen the contention that insulin and muscle work are, in addition to being quantitatively dissimilar, also qualitatively unlike. Insulin increases epididymal fat glycogen while exercise is without effect. Muscle glycogen content of different muscles was also affected differently by insulin and exercise. Insulin increased diaphragm glycogen but did not alter the glycogen content of either the triceps or gastrocnemius muscles. Exercise had no influence on diaphragm or triceps but decreased the glycogen content of the gastrocnemius muscle, which in these experiments represented the exercised muscle. These data on muscle glycogen suggest that exercise might produce a local effect in the working muscles since there was a marked difference between the exercised and resting muscles in the

same animal. Ingle[20] has also shown that while insulin decreased the plasma amino acids in eviscerate rats, exercise did not. The data on epididymal glycogen, muscle glycogen and published reports on plasma amino acids indicate that, if exercised muscles do release a humoral factor, it does not produce the same metabolic effects as insulin.

The question whether the exercise effect on glucose oxidation is local or mediated by a humoral factor was investigated by more direct means, including determination of A-V glucose differences across worked and resting legs of eviscerate dogs, and galactose accumulation in resting and exercised muscles of eviscerate rats. The data showing that exercise increased A-V glucose differences across working muscles only supports the thesis[8,23] that work effect on glucose utilization is local. This is further supported by data showing that intracellular galactose only accumulated in working muscles of eviscerate animals, and the increase in galactose was not due to an increased extracellular water since neither raffinose space nor total muscle water changed significantly in the working muscles. The results reported here do not agree with the conclusions reported by Goldstein[7] where blood from a working dog cross-transfused into a resting animal caused a depression of glucose in the recipient dog.

The mechanism responsible for the influence of exercise on glucose oxidation is, however, unknown. It is not due to temperature changes since increases in temperature which mimic that caused by work did not induce similar changes in glucose utilization. It has been suggested[18] that increased circulation in exercised muscles may be the responsible factor. The results showing that neither raffinose space nor muscle water increased in exercised muscles do not support this hypothesis.

Local hypoxia as a stimulator of glucose oxidation in exercised muscles is a possibility which remains to be investigated. Hypoxia has been found to increase glucose penetration into the cell as well as phosphorylation in an isolated heart preparation.[18] Since some degree of hypoxia probably exists in exercised muscles, it can be speculated that this may be playing a major role in the ability of work to stimulate glucose utilization. This speculation is further supported by observations showing that muscle work, as well as anoxia,[9,22] causes a depletion of muscle glycogen.

SUMMARY

Studies were described which were designed to investigate some of the changes in carbohydrate metabolism occurring during exercise and to compare these with changes produced by insulin. Experiments were also designed to test the hypothesis that exercising muscles release a humoral factor which can influence glucose utilization by resting muscles. This was done by measuring the arterial-venous glucose across resting and worked legs of eviscerate dogs and the accumulation of galactose-C-1-14 in resting and worked muscles of eviscerate rats.

Exercise or insulin was found to increase markedly glucose oxidation to C-14O_2 when uniformly labeled glucose was infused. Work had a greater effect than the doses of insulin employed. Insulin, but not muscle work, was capable of increasing glycogen synthesis by epididymal fat. While insulin increased diaphragm muscle glycogen, it did not alter the glycogen content of the triceps or gastrocnemius muscles. Muscle work, on the other hand, did not change the glycogen of the diaphragm or triceps (resting muscle) but did markedly decrease the glycogen of the gastrocnemius (working muscle). It was concluded that, although insulin and muscle work exert the same effect on blood sugar and glucose oxidation, exercise does not mimic insulin effects on fat or muscle glycogen.

Exercising one group of muscles in eviscerate dogs increased the A-V glucose difference across the worked muscles but did not change the A-V glucose difference across nonworking muscles in the same animal. Galactose-C-1-14 was found to accumulate intracellularly in worked muscles but not in nonworked muscles of the same eviscerated rat. These results were interpreted to mean that the influence of work was a local phenomenon.

Body temperatures increased during exercise by about 10° F. This was not the cause of increased glucose utilization since comparable environmentally induced body temperature changes did not result in altered glucose utilization as measured by blood sugars.

It was suggested that the local effect of work could be due to a local hypoxia since it has been shown by others that anoxia produces increased glucose utilization and depression of muscle glycogen. Comparative studies on exercise and hypoxia must be conducted to determine if hypoxia is the cause of increased glucose oxidation resulting from muscle exercise.

SUMMARIO IN INTERLINGUA

Studios Concernite con un Possibile Factor Humoral, Producite per Musculos Travaliante: Su Influentia in le Utilisation de Glucosa

Es describite studios visante a clarificar certes del alterationes in le metabolismo de hydrato de carbon occurrente in exercitio physic e a comparar los con le alterationes producite per insulina. Esseva etiam interprendite experimentos visante a testar le hypothese que musculos in exercitio emitte un factor humoral que pote influentiar le utilisation de glucosa per musculos in reposo. Isto

esseva effectuate per mesurar le glucosa arterio-venose in gambas reposante e travaliante de eviscerate canes e le accumulation de galactosa-C-1-14 in reposante e travaliante musculos de eviscerate rattos.

Esseva trovate que exercitio e insulina ha ambes le effecto de augmentar marcatemente le oxydation de glucosa a C-14O_2 post le infusion de glucosa a marcation uniforme. Exercitio habeva iste effecto a grados plus marcate que insulina in le doses usate. Insulina esseva capace a augmentar le synthese de glycogeno ab grassia epididymic. Exercitio non habeva iste capacitate. Durante que insulina augmentava le glycogeno del musculos de diaphragma, illo non alterava le contento de glycogeno in le tricipite o in le gastrocnemio. Del altere latere, travalio de musculo non alterava le glycogeno del diaphragma o del tricipite (musculo in reposo) sed reduceva marcatemente le glycogeno del gastrocnemio (musculo in travalio). Esseva concludite que — ben que insulina e travalio muscular exerce le mesme effecto in le oxydation de sucro sanguinee e glucosa — exercitio non simula le effectos de insulina in grassia o in glycogeno muscular.

Exercitio de un gruppo de musculos in eviscerate canes augmentava le differentia arterio-venose de glucosa in le musculos in travalio sed non alterava le differentia arterio-venose de glucosa in le musculos in reposo in le mesme animal. Esseva trovate que galactosa-C-14 se accumulava intracellularmente in musculos in travalio sed non in non-travaliante musculos del mesme eviscerate ratto. Iste resultatos esseva interpretate como indication que le influentia del travalio es un phenomeno local.

Le temperatura del corpore cresceva in exercitio per circa 10 F. Isto non esseva le causa del augmentate utilisation de glucosa, proque comparabile alterationes de temperatura inducite in le corpore per medios extranee non resultava in un alterate utilisation de glucosa secundo mesurationes del sucro sanguinee.

Es suggerite que le effecto local de travalio es possibilemente causate per hypoxia local, proque altere investigatores ha monstrate que anoxia produce un augmento del utilisation de glucosa e un depression del glycogeno muscular. Studios comparative in exercitio e hypoxia debe esser effectuate pro determinar si hypoxia es le causa del augmentate oxydation de glucosa que es observate post exercitio muscular.

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Method of Extraction and Bio-assay of Insulin in Blood

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A concerted effort is being made in several laboratories to find a reliable method for the measurement of insulin in blood. In the past year there have been published three extensive reviews[1-3] on the subject of plasma insulin. On reading these surveys one is confronted by the range of values, from 0 to 4,600 *u*U per ml., which has been reported for insulin levels in normal fasted individuals. The methods being used to measure micro amounts of insulin fall into three categories: in vitro bio-assay, in vivo bio-assay and immunochemical assay. The various in vitro bio-assays are based on the effect of insulin on glucose metabolism of excised tissue, such as the diaphragm of the rat[4-6] and mouse,[7] epididymal fat pad of the rat[8-10] and mammary tissue of the rat.[11] The basis of the in vivo bio-assay is a fall in blood sugar in rats[12,13] and mice [14] rendered highly sensitive to insulin by hypophysectomy. On the other hand, the immunochemical assays for insulin are based on inhibition of hemolysis of insulin-sensitized red cells,[15] competitive inhibition of binding of labeled insulin on antibody as measured by electrophoresis,[16] and a per cent change in the binding of labeled insulin to insulin antibodies in the presence of varying quantities of unlabeled insulin.[17]

The chief difficulty in the quantitation of insulin in blood is that its concentration in blood is exceedingly minute. Hastings[18] has pointed out that insulin in serum is essentially a trace protein, occurring in a molecular ratio of approximately 1:2,000,000 among other protein molecules. If one accepts, as tentative, the authors' finding of 30 *u*U of insulin per ml. of peripheral plasma in the fasting dog, the insulin concentration is 1.2 *u*g. per liter or per 70 gm. of plasma proteins, a ratio of 1:58,000,000 by weight. Another difficulty arises from the presence of factors in blood which have either insulin-like activity or anti-insulin properties. These appear to be serious drawbacks for in vitro methods of assay in which unextracted plasma is used.

The in vivo assay method to be described here is based on a fall of blood sugar in the fasted diabetic hypophysectomized (ADH) rat. Since this test animal is fairly insensitive to amounts of insulin below 500--1,000 *u*U, it has been necessary, in preparing for the assay, to extract the insulin from plasma in order to concentrate it thirty to fifty times. Inasmuch as a large amount of plasma is required for a satisfactory assay, the method is not practicable as a clinical test for insulin. The method is, however, dependable in establishing values for normal fasting plasma insulin in man and in experimental animals, and thus may serve as a reliable reference for other methods of assay. This method is being used in our laboratory to assay insulin in pancreatic vein blood of the dog in order to determine the effect of hypoglycemic compounds, such as tolbutamide, and of pituitary hormones on the release of insulin from the pancreas.

METHODS AND MATERIALS

Method of assay. The method to be described here is modified from one previously published (Anderson et al.[14]), in which the mouse was used. Male rats (Sprague-Dawley strain) six to seven weeks of age and weighing 130 to 150 gm. are hypophysectomized. Four days later a freshly prepared solution of 1 per cent alloxan monohydrate in saline is administered intravenously (40 mg./kg.). The assay is carried out eight days after hypophysectomy. It has been found recently that the hypophysectomized rat without alloxan diabetes is satisfactory as an assay animal four days after removal of the pituitary. The advantages and disadvantages in the use of the ADH rat and the hypophysectomized rat will be discussed later.

On the afternoon preceding the assay, the animals are placed in clean cages without food cups and are fed twice by stomach tube, each feeding consisting of 3 ml. of an egg-milk mixture (1 egg+250 ml. of evaporated milk). At 5 p.m. at the time of the last feeding, the animals receive 2 ml. of 6 per cent glucose in saline by

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subcutaneous injection. No more food is made available to the animals during the sixteen hours preceding the assay. On the morning of the assay the animals receive 60 mg. glucose in saline (2 ml. of 3 per cent glucose) intraperitoneally exactly one hour before the assay is started and they are then placed in a ventilated chamber warmed to 34° C. Fifty minutes later (ten minutes before the first blood specimen is taken) the rats are anesthetized with sodium pentobarbital (0.4 ml. of 0.6 per cent solution intraperitoneally). At zero time (e.g., 10 a.m.) blood is taken in duplicate specimens from the dorsal tail vein for glucose determinations and immediately thereafter the standard dose of insulin or the test material is injected into a lateral tail vein. The insulin standard is prepared in 5 per cent bovine albumin solution, and each dose is contained in 2 ml.; the dose of unknown, not more than 100 mg. of plasma extract, is made up in 2 ml. of saline; when less than 100 mg. of extract is used per dose, albumin is added to bring the protein concentration up to 5 per cent. Exactly twenty five minutes after the intravenous injection of the test material, the second blood specimen is taken in duplicate for blood glucose determinations. Glucose is determined on 0.01 ml. and 0.02 ml. of blood by the micro method of Park and Johnson.[19] The per cent fall in blood sugar over the twenty-five minute interval is taken as the measurement of insulin activity.

A four-point assay is set up using twenty to twenty-four animals with five to six animals per group. Two dose levels of insulin standard (glucagon-free insulin, Lilly), e.g., 2,000 *u*U and 6,000 *u*U, or 3,000 *u*U and 9,000 *u*U are given, and two levels of the unknown in quantities having a 1:3 ratio.

Extraction of insulin from plasma. The pilot method for the extraction of insulin from plasma was based on a percolation method previously developed by Bates et al.[20] for the extraction of thyrotrophin from lyophilized plasma. The information gained from this procedure allowed for the development of a routine method for the extraction of insulin from plasma. Having established the optimal conditions for extraction, percolation was no longer used. Instead the extractions were made in flasks and the extracts separated by centrifugation.

The two methods, namely, percolation and centrifugation, are as follows: In the *percolation method,* a batch of approximately 7.5 gm. of lyophilized plasma which has been through a forty to sixty mesh screen of a Wiley mill is mixed dry with 7.5 gm. of a filter aid (Hyflo) and suspended in 100 ml. of 95 per cent ethanol. This mixture is transferred to a small Buchner funnel with a sintered glass plate which has been precoated with Hyflo. The percolate is collected in graduated tubes placed in a 500 ml. suction flask. The percolate from the 95 per cent ethanol is discarded; next 100 ml. of 76 per cent ethanol solution is allowed to percolate through the funnel. This is followed in stepwise sequence with 100 ml. of 76 per cent ethanol with 2 per cent NaCl, 100 ml. of 67 per cent ethanol with 5 per cent NaCl, and 100 ml. of 57 per cent ethanol with 7 per cent NaCl. The percolates are collected in 25 ml. fractions. Initially the specific gravity of each fraction was determined and the absorbancy at 276 mu was measured in order to obtain information as to pooling the fractions. The pools are concentrated in a flash evaporator to remove ethanol, then dialyzed overnight against cold running tap water to remove NaCl, and finally lyophilized and weighed.

In the *centrifugation method,* a batch of 7.5 gm. of lyophilized milled plasma is mixed with one third of its weight with Hyflo in a 500 ml. centrifuge bottle and extraction carried out with the following solutions in sequence: 95 per cent ethanol; 76 per cent ethanol—2 per cent NaCl and 57 per cent ethanol—7 per cent NaCl. The 95 per cent ethanol supernatant is discarded. The other supernatant solutions are pooled and concentrated in the flash evaporator to remove the alcohol, dialyzed against cold running tap water to remove the NaCl, and finally lyophilized.

The centrifugation method has been modified by extracting plasma without previous lyophilization. The method is as follows: 100 ml. plasma is mixed with 8 gm. NaCl and 4 gm. Hyflo and placed on a magnetic stirrer which is set at a low speed; 400 ml. of 95 per cent alcohol is added and the mixture is left on the stirrer for thirty minutes to complete denaturation of most of the plasma proteins. The batch is then centrifuged, the residue resuspended in 300 ml. of 57 per cent ethanol containing 7 per cent NaCl and extracted as before. The supernatant fractions from the two extractions are pooled, concentrated in the flash evaporator to remove the alcohol, dialyzed against cold running tap water overnight and then lyophilized. The lyophilized extract is mixed with an equal amount of Hyflo and extracted with 95 per cent ethanol (50 ml. per gram of dry material). After centrifugation the supernatant is discarded and the residue extracted once more with 95 per cent ethanol. This lipid-free residue is then extracted three times with water (30 to 40 ml. each time). The three extracts are pooled, lyophilized, and weighed. This last method has been found to be the most satisfactory, and is the one currently used.

RESULTS

Observations on the assay method. The log dose-response curve for insulin is shown in figure 1, which is a composite of the data on the insulin standard curves obtained with the alloxan diabetes hypophysectomized (ADH) rat. Each observed point is the mean value obtained from the number of animals given in the parentheses, and the standard error of the mean is indicated by the vertical bar. The biological response, namely, the per cent fall in blood sugar, is clearly a linear function of the log-dose in the range of 1,000 *u*U to 9,000 *u*U. The index of precision (λ) is 0.36. The log-dose response curve for the hypophysectomized rat is practically identical to that given in figure 1, but the standard errors are less.

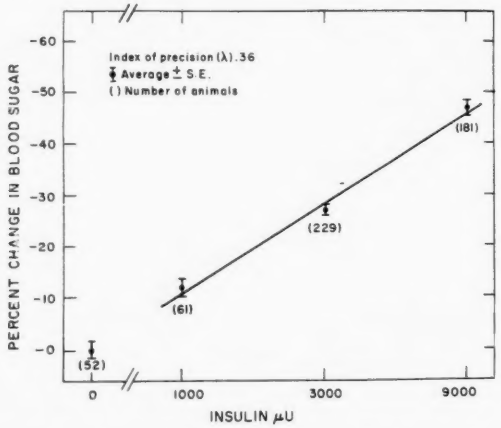

FIG. 1 Change in blood sugar for different doses of insulin in the alloxan diabetic hypophysectomized (ADH) rat.

An illustration of a routine four-point assay is shown in figure 2. Twenty animals were used in the assay with five rats to a group. Two groups were given standard insulin, 3,000 and 9,000 *u*U respectively, and two groups, extract RB9-73, tubes 5-8 (extract of plasma containing insulin I^{131}), in doses of 1 mg. and 3 mg. respectively. It will be seen in figure 2 that the regression lines for the standard and the unknown are essentially parallel. By graphical interpolation the assay showed 1,650 *u*U/mg. as based on the 1.0 mg. dose and 1,583 *u*U/mg. as based on the 3.0 mg. dose, or a mean value of 1,616 *u*U/mg.

Our experience with four-point assays over a period of twenty-one months is summarized in table 1. The data indicate a satisfactory correlation of potency at the two dose levels. Further evidence of the reliability of

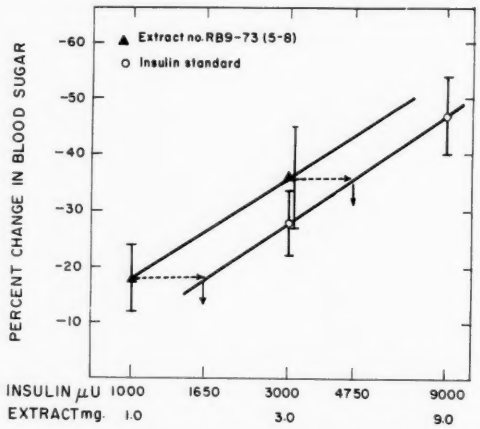

FIG. 2. Four-point assay of extract of plasma containing insulin I^{131}. By interpolation on standard curve 1.0 mg. of extract contains 1,650 μU and 3.0 mg. 4,750 μU.

TABLE 1

Summary of four-point assays of insulin

Date of assay	Plasma fraction	Insulin μU/mg. Based on amounts of extract x	3x
11-18-58	*RB9-73	127	133
12-17-58	*RB9-73, tubes 9-15	1,450	1,266
12-19-58	*RB9-73, tubes 5-8	1,650	1,583
1-6-59	*RB9-75	1,500	1,111
1-22-59	*Plasma + insulin	1,600	2,200
3-10-59	W2-27	291	258
4-28-59	RB9-128	147	153
5-12-59	Repeat on RB9-128	133	144
6-23-59	RB9-119A	260	224
9-17-59	RB9-110	1,047	1,196
9-24-59	*Albumin 5% + insulin	1,833	1,666
11-5-59	RB12-151B	424	255
11-19-59	*Albumin 5% + insulin	43	32
12-3-59	*RB12-148A	87	100
12-10-59	*RB12-146B	1,142	1,242
6-9-60	W5-4	274	270
7-28-60	W6-15	360	253

*Insulin I^{131} present.

the bio-assay method is illustrated in table 2, in which the bio-assay is compared with the radioactive count carried out on fractions of extracted plasma to which insulin I^{131} had been added. The two methods of quantitative measurement show very close correlation.

Observations on extraction procedures. The amount of lyophilized extract obtained from plasma varies between 1 and 2 per cent by weight of the original solids in the plasma, depending upon the method used.

In three experiments insulin I^{131} was added to the plasma and recovery of insulin checked by radioactive count and by bio-assay. The data from one experiment in

TABLE 2

Comparison of insulin measurements by bio-assay and radioactivity

Plasma fraction	Per cent of insulin recovered as measured by Bio-assay	I^{131} activity
RB9-73	64	78
RB9-75	74	77
RB12-148A } RB12-148B }	91	88
RB12-146A } RB12-146B }	76	82

which lyophilized plasma (Fraction RB-73) containing insulin I^{131} was extracted by the percolation procedure are shown in figure 3 and in table 1. The activity of the starting material containing the labeled insulin (Abbott Insulin I^{131}) was measured both by radioactive count and by bio-assay before starting the extraction. The 76 per cent fraction (RB9-73 tubes 5-8) contained 41 per cent of the added insulin measured by the radioactive count or 36 per cent by bio-assay. The combined fractions of 76 per cent ethanol—2 per cent NaCl and 67 per cent ethanol—5 per cent NaCl (RB9-73 tubes 9-15) contained 33.6 per cent of the added insulin by radioactive count or 29 per cent by bio-assay. In another experiment, in which insulin I^{131} was added to lyophilized plasma and the extraction carried out using centrifugation instead of percolation, the insulin recovered (Fraction RB9-75, table 2) was 74 per cent as measured by radioactive count and 77 per cent by bio-assay.

A recovery experiment in which insulin I^{131} was added to neat plasma and extracted directly without first lyophilization is shown in table 3. To 670 ml. of fasted peripheral dog plasma, which was estimated to contain

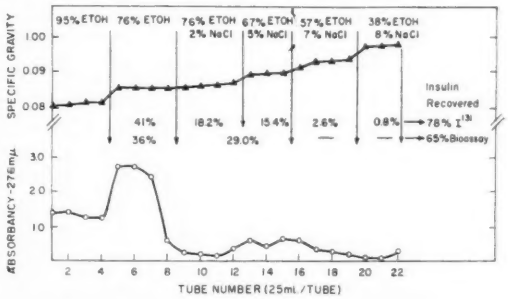

FIG. 3. Sequence of insulin fractions in extraction of plasma by percolation. Assays of fractions are given in table 1. Starting material, lyophilized plasma (RB9-73) 7.5 gm.; 76 per cent ETOH extract (RB9-73 tubes 5-8), 75 mg.; combined extracts 76 per cent ETOH—2 per cent NaCl and 67 per cent ETOH—5 per cent NaCl (RB9-73 tubes 9-15), 52 mg.

20,000 μU endogenous insulin (30 μU per ml.) was added 170,000 μU labeled insulin (Abbott Insulin I^{131}) which had been assayed in our laboratory. It will be seen in table 3 (Fractions RB12-148A and B) that 91 per cent of the insulin was recovered as measured by bio-assay and 88 per cent by radioactive count. With refractionation of the combined fractions RB12-148A and B the recovery by bio-assay was 76 per cent and by radioactive count 82 per cent.

DISCUSSION

In connection with the bio-assay procedure it was mentioned that the hypophysectomized rat may be used instead of the alloxan diabetic hypophysectomized (ADH) rat. The advantages of one as against the other are as follows: With hypophysectomy alone the time and work involved are considerably less and the rat mortality

TABLE 3

Recovery of insulin I^{131} added to plasma

Starting material		Weight g.	Recovery Bio-assay μU	Bio-assay Per cent	Radioactivity Per cent
Plasma (dog, fasting) 670 ml. containing insulin 20,000 μU* Insulin I^{131} 170,000 μU†	RB12-148A (76% alc.—2% NaCl)	1.8	172,980	91.0	80.0
	RB12-148B (57% alc.—7% NaCl)	0.34	0	0	7.9
Total starting: 190,000 μU	Total recovery	2.14	172,980	91.0	87.9
1.38 g. Fraction RB12-148A refractionated	Tubes 4-11	0.335	21,400	16.9	21.0
	Tubes 12-19	0.063	75,096	59.4	61.0
Total starting: 126,480 μU	Total recovery	0.398	96,496	76.3	82.0

*Estimated endogenous insulin on basis of 30 μU/ml. †Bio-assay of I^{131} insulin before added to plasma.

is almost nil. Moreover, the blood sugar of hypophysectomized rats shows a remarkable uniformity after a seventeen-hour fast in contrast to that of the ADH rats, in which the blood sugar is higher, but much more variable. These variations increase the standard error in the assay. The advantages in using the ADH rat are that a higher fasting blood sugar permits a wider range of insulin dosage in the assay, and the possible release of endogenous insulin during the assay is excluded. Hypophysectomized rats may be used for multiple assays, but the best assay animal is one taken three to ten days after removal of the pituitary. The fasting blood sugar level becomes progressively lower with time and this limits the range of insulin dosage. It should be stressed that the assay animal must be fasted.

In the recovery experiments in which labeled insulin was used a remarkably high percentage of insulin was extracted. We are well aware that Crystalline Insulin added to plasma may be more readily extracted than endogenous insulin and that this may account for the high percentage of recovery. The question whether insulin inhibitors or insulin-like substances are in the insulin fraction after extraction, cannot be answered by our studies. If there are substances other than insulin which lower the blood sugar under the conditions of our in vivo assay, they are unknown to us. Glucagon, which is known to raise blood sugar by releasing glucose from liver glycogen, has been found by us to have no "anti-insulin" effect in the fasted ADH animal.

SUMMARY

A method for the assay of insulin has been described using the fasting hypophysectomized alloxan diabetic rat or the hypophysectomized rat as the test animal. The per cent fall in blood sugar over a twenty-five-minute period following the intravenous injection of the material to be assayed is taken as the measurement of insulin activity. This has been shown to be a linear function of the log-dose of insulin in the range of 1,000 *u*U to 9,000 *u*U. The index of precision (λ) is 0.36. A four-point assay is used with four to six animals per group. The accumulated data over several months indicate a high correlation between the two dose levels. Comparison of insulin measurements by radioactive count and bio-assay also reveals highly satisfactory agreement.

Methods for the extraction of insulin from plasma with a fiftyfold concentration of activity using ethanol solutions containing NaCl are described. Recovery of insulin added to plasma, checked by radioactive count, was 77 to 88 per cent and by bio-assay 65 to 91 per cent.

SUMMARIO IN INTERLINGUA

Methodo pro le Extraction e le Bio-Essayage de Insulina in Sanguine

Es describite un methodo pro le essayage de insulina que utilisa como animales experimental hypophysectomisate rattos con diabete per alloxano in stato jejun o rattos mermente hypophysectomisate. Le procentage del reduction in le nivello del sucro sanguinee occurrente intra vinti-cinque minutas post le injection intravenose del material sub investigation es acceptate como mesura del activitate de insulina. Ha essite trovate que isto es un function linear del logarithmo del dose de insulina in le region inter 1.000 e 9.000 *u*U. Le indice de precision es 0,36. Un essayage a quatro punctos es usate, con quatro a sex animales in omne gruppo. Le datos accumulate in le curso de plure menses reflecte un alte correlation inter le duo nivellos de dose. Le comparation del determination de insulina per contation de radioactivitate e per bio-essayage etiam revela un satisfacentissime accordo.

Es describite methodos de extraction de insulina ab plasma que resulta in un cinquantuple concentration de activitate e utilisa solutiones de ethanol a contento de NaCl. Le redetection de insulina addite al plasma amontava a inter 77 e 88 pro cento a base del contation de radioactivitate e a inter 65 e 91 pro cento a base del bio-essayage.

ACKNOWLEDGMENT

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Linoleic Ester and Cholesterol

In only a few cases have experiments been reported testing the effects of individual fatty acids on cholesterol levels. The authors of this study have used a highly purified preparation of ethyl linoleate in order to circumvent the problem of interpretation arising from using fat preparations of unknown or complex composition (F. W. Quackenbush and M. D. Pawlowski *J. Nutrition* *72*:196, 1960).

Using fractional crystallization of urea addition compounds followed by low pressure distillation the authors prepared a *cis* 9, 12 linoleic ester at least 97 per cent pure. The diets were either 20 per cent or 2 per cent fat which was either hydrogenated coconut oil (HCO) or soybean oil (SBO). Cholesterol was 1 per cent and sodium glycocholate was 0.5 per cent of the diet. Casein was 18 per cent of the diet.

Weanling male and female rats were used. The first three groups had twelve males and twelve females in each group and there were eight males each in groups four, five, and six.

Group one was fed the 20 per cent soybean oil diet. Group two, receiving a 20 per cent hydrogenated coconut oil diet, was divided at the end of five weeks so that half the animals of each sex were fed 10 per cent of HCO and 10 per cent of linoleic ester. The third group (2 per cent HCO) was divided at the end of five weeks so that half the animals of each sex were given 2 per cent linoleic ester instead of HCO. Groups four, five, and six were fed 2 per cent HCO through five weeks. Group four was then given 2 per cent SBO and group five was given 4 per cent SBO. Group six received a 25 mg. supplement of linoleic ester daily to the 2 per cent HCO diet.

Plasma was analyzed for free and total cholesterol, and liver and carcass were analyzed for total lipid and cholesterol.

Animals receiving the hydrogenated coconut oil diets gained substantially less weight than those getting the soybean oil ration, but later replacement of half the HCO with linoleic ester improved the growth. Essential fatty acid deficiency evidenced by scaliness of paws and tails appeared by the third week on the HCO diet and continued through the experiment, being more pronounced in males.

Plasma cholesterol levels averaged about 50 mg. per 100 ml. in weanling rats with about 80 per cent as ester cholesterol. In some cases the animals getting high or low fat HCO diet for four weeks had total plasma cholesterol values as high as 1,000 mg. per 100 ml. in contrast to soybean oil animals averaging about 200 mg. per 100 ml.

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Rates of Production, Utilization, Accumulation and Apparent Distribution Space of Glucose

Effects of Insulin in Dogs Using a Validated Tracer Method

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Various tracer methods have been proposed to determine the rate of appearance, frequently termed the turnover rate, of a selected substance in the blood plasma through application of the dilution principle. The accuracy and applicability of most of these methods depend on a number of assumptions regarding the dynamic state and the pattern of distribution in the body of the selected metabolite. These assumptions have frequently been left unstated in publications on the subject. With the exception of the method of successive tracer injections[1] none of the published tracer methods for calculating rates of production and utilization has been validated in vivo against absolute measurements.[2,3]

In this investigation the effects of insulin on the amount and the rates of appearance and disappearance of glucose in the central compartment, of which blood plasma is a representative part, have been studied using the method of successive tracer injections. As no chemical transformation of glucose takes place in the plasma the above rates represent rates of transfer of glucose into and out of the central distributing compartment. The rate of production (appearance) of glucose in this compartment probably represents the rate of glucose output by the liver under our experimental conditions and perhaps a very small contribution by other tissues. The rate of disappearance of glucose from this compartment represents the rate of glucose uptake (utilization) by all tissues outside the central mammillary compartment, and includes the rate of glucose excretion if such is present. The steps followed in calculating the intermixing amount and rate of appearance of glucose at each time of tracer injection are the same as those described and used in establishing the validity of the method.[2,3]

It is also possible to calculate the amount (N) of rapidly intermixing glucose (termed the *glucose mass*) and the *apparent glucose space* $V = N/C$ at each time of intravascular tracer injection, where C is the plasma glucose concentration. It is emphasized, however, that V is not required for the calculation of rates of transfer of glucose into and out of the central compartment if the method of successive tracer injections is used.

MATERIALS AND METHODS

a. *Animals*

The experiments were performed on normal and depancreatized dogs under Nembutal anesthesia. Removal of the pancreas was carried out six to twenty-one days prior to an experiment. The depancreatized animals were fed a diet containing raw pancreas. Crystalline Insulin was injected twice daily. The last injection of insulin was given forty-eight to sixty hours before the experiment.

b. *Experimental technics*

Glucose uniformly labeled with C-14 was injected intravenously via an indwelling plastic catheter inserted about three to six inches into the cephalic vein. When a constant infusion was administered, it was given through another plastic catheter inserted *via* the saphenous into the iliac vein. Samples of mixed venous blood were withdrawn through a third catheter from either the inferior vena cava or from the right auricle of the heart. The latter was catheterized via the left jugular vein. Immediately after being withdrawn the blood samples were mixed with sodium fluoride and dry heparin, cooled in an ice water bath and centrifuged within one hour.

For the infusion of solutions a standard infusion pump was used (Harvard Apparatus Co., Dover, Mass., U.S.A.). The amount of fluid delivered was 0.38 ml./min. In experiments 3, 4 and 7 the infusion was given from a calibrated Marriotte burette delivering 0.5 ml./min.

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c. *Chemical methods and determination of specific activity.*

Plasma was deproteinized according to Somogyi.[4] The concentration of glucose was determined in an aliquot of the filtrate by the method of Nelson.[5,6] Glucosazones were prepared by the standard procedure[7] from the rest of the filtrate after a measured amount of carrier glucose had been added. The glucosazones were transcrystallized from boiling 60 per cent ethanol, plated on preweighed filter paper circles and were counted in a windowless flow counter. Radioactive strengths of all samples, recorded as net counts per minute (c.p.m.), were corrected for self-absorption. The specific activities of plasma glucose samples were expressed as c.p.m./mg. of glucose carbon.

d. *Experimental design*

In most experiments five successive injections of glucose uniformly labeled with C-14 were given, with 15-30 μC of C-14 being administered per injection. The start of the infusion or the injection of insulin was made between the second and third injections. Thus two sets of calculated values for glucose production and utilization and the glucose mass were obtained before infusion of insulin and three afterward. A sixth value for the glucose mass was calculated at the time of the start of the insulin infusion as the product of the average apparent glucose space in the pre-insulin period and the concentration of plasma glucose at the start of the infusion of insulin.

The hepatic glucose output was also calculated immediately after insulin by extrapolating the specific activity versus time curve back to the time when the infusion of insulin was started, using the formulae given by Henderson et al.[7] In contrast to the rates calculated by the method of successive tracer injections, the validity of the rate of appearance calculated from the slope of this curve rests on the additional assumption that the apparent glucose space did not change abruptly in magnitude at this time. The validity of this assumption has been verified previously[1,8] and again in this study (table 2).

e. *Curve fitting*

The course of the specific activity of plasma glucose, plotted as a function of the time, appeared by inspection to follow a single exponential function when an initial intermixing time of fifteen minutes was allowed. This function was fitted to each set of data using the method of least squares.

A polynomial function was fitted to interrelate the calculated glucose masses and the times at which they were determined for the calculation of the rates of glucose utilization. Separate functions were fitted to pre-

TABLE 1

Spontaneous variation of the rates of glucose production (R_A) and utilization (R_D) in one normal and one depancreatized dog. Rates are calculated at tracer injection times and are expressed as mg. glucose/kg. body weight/min. t = 0 at the time of the first injection.

Tracer inj. time (min.)	0		60		120-125		165		180		210	
Rates:	R_A	R_D	R_A	R_D	R_A	R_D	R_A	R_D	R_A	R_D	R_A	R_D
Normal dog	2.87	3.16	2.41	2.68	3.39	3.64	4.83	5.07	—	—	3.60	3.83
Depancreatized dog	7.76	7.79	10.63	10.66	11.94	11.96	—	—	8.78	8.78	—	—

TABLE 2

Effect of insulin on plasma glucose level, C, (in mg. per 100 ml.), glucose mass, N, (gm.) and apparent glucose space, V, (in per cent of body weight) in normal dogs

No. of expt.	Wt. of dog kg.	Mode of administration of insulin	Before insulin: 100 min. N	V	C	40 min. N	V	C	Times of injection of tracer glucose after injection or start of infusion of insulin: 23-25 min. N	V	C	60-69 min. N	V	C	108-114 min. N	V	C
1*	12.0	Inject. 1.0 U/kg.	2.41	17.3	115.7	2.02	15.7	106.9	—	—	—	1.69	46.8	30.0	—	—	—
7A	23.6	Inject. 0.3 U/kg.	3.58	18.0	84.2	2.94	14.7	84.2	2.73	44.1	26.2	3.42	33.3	43.5	4.85	24.1	85.4
3	11.5	Infus. 8 mU/kg./min.	2.40	23.4	89.1	—	—	—	1.77	24.3	63.2	1.60†	44.4†	31.4	1.34	30.3	38.7
6	16.9	Infus. 3.8 mU/kg./min.	3.56	19.8	106.2	3.67	22.3	97.4	1.65	17.7	55.1	10.23†	182.3†	33.2	1.59	29.0	32.5
7	25.0	Infus. 1.9 mU/kg./min.	4.23	21.8	77.8	4.21	21.6	77.8	3.35	20.9	64.0	4.31	31.0	55.5	2.26	32.0	28.2

*In this experiment the first two injections of tracer were given 120 and 60 min. before insulin; the third tracer injection was given simultaneously with insulin. N was found to be 1.42 gm.; and V 12.5 per cent with this last injection of tracer.

†These determinations are based on a specific activity vs. time curve of a very poor fit. Therefore it may represent a gross overestimation of N and V. They are included in order to present unselected data.

insulin and post-insulin data. The glucose mass was determined by extrapolation of each specific activity versus time curve back to the time of tracer injection for each of the five injections of tracer. A sixth value was calculated as described in (d) above. The rates of changes of the glucose mass were calculated as the derivative of the above-mentioned polynomial function at times of tracer injections. All curve fittings were performed by an IBM 650 digital computer.

f. *Materials*

Glucose uniformly labeled with C-14 was used (Merck and Co., Montreal, Canada). The injected solution contained 5 mg. per ml. glucose corresponding to an activity of 5 μC. Glucose of analytical grade (Analar BDH) was used for infusion of nonlabeled glucose. Glucagon-free insulin (Lilly, Lot 499667) was used in all but two experiments. A fresh acid-aqueous solution of the insulin was prepared before each of these experiments. In experiments 1 and 4 Insulin-Toronto was used.

RESULTS AND DISCUSSION

1. *Control experiments*

The glucose mass, the apparent glucose space and the rates of output and utilization of glucose, were calculated in two experiments in which no insulin was given. Figure 1 shows the spontaneous variation of the glucose mass and the apparent glucose space in a normal dog. A similar experiment yielding essentially the same results for a depancreatized dog was reported earlier.[9]

The spontaneous variations of the rates of hepatic glucose output and utilization are shown in table 1 for these two dogs. Although rates of transfer vary within ± 41 per cent in the normal and ± 23 per cent in the depancreatized dog around their respective mean values, no particular trend is detectable in their variation.

2. *Effects of insulin in normal dogs*

In a preliminary report it was shown that there is a delayed increase, after either the injection or infusion of insulin, in the apparent glucose space.[8] This is illustrated in table 2. The rates of glucose transfer calculated at injection times and at the time of the start of the infusion of insulin are shown in tables 3 and 4. These data and calculations indicate that, after a short depression, the rate of hepatic glucose output increases during the infusion of insulin, at a time when the plasma glucose level is low. In each case a considerable increase in the rate of utilization was observed throughout the first seventy minutes of the infusion or after injection of insulin. Later during hypoglycemia both rates decreased.

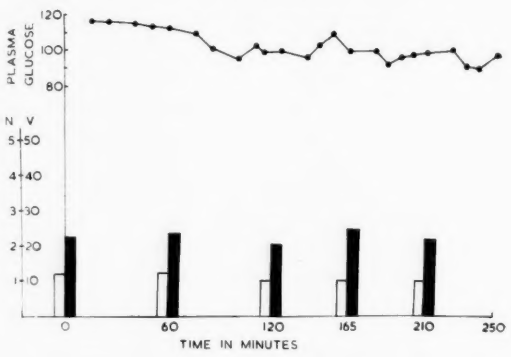

FIG. 1. Spontaneous variation with time of plasma glucose level (as mg. per 100 ml.), the amount of rapidly intermixing glucose (N, in grams) and apparent glucose space (V, as per cent of body weight) in a normal anesthetized dog. Upper line: plasma glucose level; white columns: the amount of rapidly intermixing glucose; black columns: apparent glucose space.

3. *Effects of simultaneous infusion of insulin and glucose in normal dogs*

Insulin caused severe hypoglycemia in the above-mentioned experiments. In order to separate the effects of insulin from those of the hypoglycemia caused by it glucose and insulin were infused simultaneously in three normal dogs in an effort to maintain the blood glucose at a constant level. In one of these three experiments the amount of glucose was insufficient to prevent hypoglycemia. As shown in table 5 the increase of the apparent glucose space after insulin was obtained even in the absence of hypoglycemia. Calculated rates of hepatic glucose production are shown in table 6. This rate was computed as the difference between the tracer-calculated rate of glucose appearance and the measured rate at which glucose was infused. It is evident that even in the absence of hypoglycemia the rate of hepatic glucose production may increase, but the increase was only transient in one of the two experiments. On the other hand, the rate of glucose utilization was consistently greater when hypoglycemia was avoided (compare tables 4 and 6).

4. *Effects of insulin in depancreatized dogs*

The effects of insulin infusion on the intermixing mass of glucose, apparent glucose space and the plasma concentration of glucose in three insulin-deprived depancreatized dogs are shown in table 7. In these diabetic animals it can be seen that, in contrast to the normals, insulin did not increase the apparent glucose space. The rate of hepatic glucose output either was not increased at all during the insulin infusion, or only transiently and slightly. In the second hour of the infusion the cal-

TABLE 3

The effect of insulin on the rate of glucose production in normal dogs. Rates are expressed as mg. glucose/kg. body weight/min. The magnitude of rates printed in italics are not calculated by the method of successive tracer injections but from the slope of the specific activity vs. time curve of plasma glucose at the indicated time. (See text.) The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 2.

No. of expt.	Time before insulin (min.)				Time after injection or infusion of insulin (min.)				Mode of administration of insulin
	120	100	60	40	0	23-25	60-69	108-114	
1	1.91	—	2.82	—	4.73*	—	5.34	—	INJ. 1 U/kg.
7A	—	1.71	—	2.70	*0.09*	9.08	4.05	3.28	INJ. 0.3 U/kg.
3	—	2.06	—	2.51	*0.94*	4.50	6.84	5.12	INF. 8.0 mU/kg./min.
6	—	2.36	—	4.13	*0*	6.72	20.76	4.44	INF. 3.8 mU/kg./min.
7	—	2.33	—	4.19	*1.00*	8.70	6.65	3.82	INF. 1.9 mU/kg./min.

*Determined by the extrapolation of ln specific activity vs. time curve to tracer injection time. Insulin and tracer glucose were injected simultaneously at t = 0; blood samples were collected between 15 and 60 min. after injection.

TABLE 4

Effect of insulin on the rate of the utilization of glucose in normal dogs. Rates are expressed as mg. glucose/kg. body weight/min. See legend to table 3 concerning figures in italics. The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 2.

No. of expt.	Time before insulin (min.)		Time after the injection or the start of the infusion of insulin (min.)				Mode of administration of insulin
	100	40	0	23-25	60-69	108-114	
7A	2.24	3.54	*6.29*	9.80	4.45	2.29	INJ. 0.3 U/kg.
3	2.47	2.20	*4.07*	4.96	7.30	5.58	INF. 8.0 mU/kg./min.
6	2.37	4.14	*5.70*	10.32	21.01	0.92	INF. 3.8 mU/kg./min.
7	3.05	4.14	*4.06*	8.78	7.28	5.54	INF. 1.9 mU/kg./min.

TABLE 5

Effect of the simultaneous infusion of insulin and glucose on the plasma glucose level C (mg. per cent), glucose mass N (gm.) and apparent glucose space V (in per cent of body weight) in normal dogs

No. of expt.	Infused amount of Glucose mg./kg./min.	Insulin mU/kg./min.	Before Infusion 100 min. N	V	C	40 min. N	V	C	During infusion 20-25 min. N	V	C	60-65 min. N	V	C	110 min. N	V	C
9	5.3	5.3	2.86	31.9	78.2	3.14	35.6	78.2	3.70	52.0	63.0	2.05	64.7	28.0	3.26	160.1	18.0
10	10.5	3.1	3.05	24.0	82.7	2.86	22.5	82.7	7.54	33.0	148.2	8.76	51.7	110.1	4.78	28.8	108.0
11	8.4	3.2	3.22	27.8	77.8	3.21	26.7	77.7	5.58	44.6	84.0	4.46	48.2	62.2	2.92	36.6	53.6

TABLE 6

The effect of the infusion of insulin plus glucose on the rates of glucose production (R_A) and utilization (R_D) in normal dogs. Rates are expressed as mg. glucose/kg. body weight/min. The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 5.

No. of expt.	Infusion rate of glucose mg./kg./min.	insulin mU/kg./min.	Before infusion 100 min. R_A	R_D	40 min. R_A	R_D	During infusion 20-25 min. R_A	R_D	60-65 min. R_A	R_D	110 min. R_A	R_D
9*	5.3	5.3	3.37	2.90	4.82	4.35	10.09	16.73	6.96	15.80	12.31	6.23
10	10.5	3.1	2.39	2.19	3.52	3.35	6.92	9.87	16.88	28.66	2.61	25.30
11	8.4	3.2	2.52	2.52	3.48	3.48	7.40	17.80	3.49	17.89	0.48	11.50

*In this experiment the amount of glucose infused was insufficient to prevent hypoglycemia. The plasma glucose level fell to 18 mg. per cent 110 min. after infusion had been started.

culated rate of glucose output dropped to the pre-insulin level. The rates of glucose utilization increased very considerably during insulin infusion (table 8).

Considering all the above results it appears that in *normal* dogs insulin increased the apparent distribution space of glucose. The initial reduction of the level of plasma glucose was due mainly to an early increase in the rate of utilization of glucose from the plasma compartment. The rate of output of glucose into this compartment was transiently depressed following insulin administration. Later it increased above the pre-insulin level. This increase, however, did not exceed the enhanced rate of peripheral utilization, and the plasma glucose level remained low. When hypoglycemia was prevented by the simultaneous infusion of glucose with insulin the rate of glucose utilization rose to very high levels while the increase in the rate of hepatic glucose output appeared to be less than when hypoglycemia was permitted. The increase in the apparent glucose space was still observed under this condition. In the *depancreatized* dogs the apparent glucose space was not increased by insulin and no compensatory elevation of the rate of appearance of glucose was observed during the infusion of insulin. In other respects the response of depancreatized dogs was similar to that of normal animals.

Changes in the apparent glucose space could arise from alterations in the relative concentrations of the glucose distributed between the different parts of the extra-cellular fluid volume. The possibility that the observed increase of the apparent glucose space after insulin is due to such a shift in glucose concentration is rendered unlikely by the increase in glucose utilization after insulin in the peripheral tissues. This increased utilization widens the difference between the concentrations of glucose in the blood plasma and in the interstitial fluid. Therefore the calculated value of the apparent glucose space, based on the concentration of glucose *in the blood plasma,* would tend to be reduced after insulin. In fact, however, the apparent glucose space in normal dogs is *increased* by insulin.

The increase of the apparent glucose space is thus due to the addition of glucose, probably that within cells, to the rapidly intermixing mass of glucose *without a corresponding increase in plasma glucose concentration.* The fact that the calculated amount of intermixing glucose exceeds the estimated amount of extracellular glucose even in the absence of excess insulin[10] indicates that some part of the rapidly intermixing glucose is intracellular. The difference between the calculated glucose mass and the amount of extracellular glucose is roughly the quantity of glucose which one would expect to find as free glucose in the liver.[11] It is postulated here that insulin acts by adding more *intracellular* glucose to the glucose mass. This may mean either an actual increase in the volume of fluid in which glucose is distributed — as predicted by the "permeability hypothesis" of insulin action[12] — or an increase in the concentration of glucose

TABLE 7

Effect of the infusion of insulin on the glucose mass, N (gm.), the apparent glucose space, V (in per cent of body weight) and the plasma glucose level, C (mg. per cent) in depancreatized dogs

No. of expt.	Amount of insulin infused mU/kg./min.	Before infusion 100 min. N	V	C	40 min. N	V	C	During infusion 25 min. N	V	C	65 min. N	V	C	110 min. N	V	C
12	6.3	14.90	24.7	398	16.28	26.9	398	11.15	20.9	350	8.33	18.6	294	7.76	26.0	196
13	6.3	12.00	25.5	389	12.67	29.6	353	7.41	25.2	243	5.62	23.4	198	4.22	25.1	139
14	6.1	9.10	24.0	283	8.40	22.5	279	7.51	25.4	221	6.79	23.4	217	3.25	17.1	142

TABLE 8

The effect of the intravenous infusion of insulin on the rates of glucose production (R_A) and utilization (R_D) in depancreatized dogs. Rates are expressed as mg. glucose/kg. body weight/min. See legend to table 3 concerning figures in italics. The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 7.

No. of expt.	Rate of infusion of insulin (mU/kg./min.)	Before infusion 100 min. R_A	R_D	40 min. R_A	R_D	During infusion 0 min. R_A	R_D	25 min. R_A	R_D	65 min. R_A	R_D	110 min. R_A	R_D
12	6.3	6.79	5.77	8.99	7.91	*≈0*	*18.90*	10.58	18.48	9.47	10.74	9.36	11.68
13	6.3	7.44	5.38	8.62	8.84	*1.91*	*24.96*	14.88	24.46	8.29	8.63	9.04	17.03
14	6.1	3.80	4.75	7.27	8.21	*0*	*1.50*	4.76	5.42	4.93	7.62	4.20	14.12

in those intracellular compartments in which intermixing glucose is distributed even in the absence of insulin or of an excess of insulin. If the first possibility should be the only mechanism of the action of insulin, it would be expected that the apparent glucose space would increase in both normal and depancreatized dogs, as both animals responded well to insulin. This increase, however, could not be demonstrated in depancreatized dogs. To account for the difference it is suggested that free hepatic glucose originating from glycogen may play a role. Hepatic glucose is presumably a part of the amount of rapidly intermixing glucose.[13] Insulin results in a breakdown of glycogen in the liver in normal animals[14,15] even when glucose is administered.[16,17] This glycogen may be converted at least partly to free intracellular glucose, which in terms of the preceding hypothesis would contribute to the calculated amount of rapidly intermixing glucose. In insulin-deprived depancreatized dogs the glycogen content of the liver is low, the effect of insulin being definitely glycogenic.[18] Therefore little or no increase in free glucose and thus in the apparent glucose space would be expected. There is no evidence at present to support this suggestion. On the other hand it is possible that the difference in the responses of normal and diabetic dogs is due to the fact that the plasma glucose did not reach as low levels in the latter group as in the former.

It must be recognized that, if the intracellular hepatic glucose is part of the rapidly intermixing glucose in the body, the calculated rate of glucose production will be higher than the actual hepatic glucose output whenever glycogen is breaking down at a faster rate to intracellular glucose than the latter is released from the liver. Further studies of the role of the liver in the mechanism of the increase of the apparent glucose space are in progress.

In most of the normal dogs tested, insulin was found to cause a sudden and transient decrease in the rate of production of glucose. As the evidence for this was based on the shape of the fitted specific activity versus time curves its validity and accuracy remain questionable (see "Methods" in this paper and references 1, 19). No such reservations are necessary when we consider the rates of glucose production calculated at the times of tracer injection.[1] Rates of hepatic glucose output calculated after the infusion of insulin had proceeded for about twenty-five minutes were markedly elevated. These later decreased slowly toward pre-insulin values. The output of glucose did not decrease during the infusion of insulin as described by de Bodo et al.[20] These workers, using the method of Wall et al.,[21] had to assume that the apparent glucose space remained constant, a premise which now seems to lack adequate support.[8] This assumption would lead to an underestimation of the rate of glucose production.

When hypoglycemia was prevented insulin still tended to increase the rate of hepatic glucose production. This increase, however, was confined to the first hour of the infusion, and was small in one of the two experiments. It is conceivable that this increase is due to a breakdown of glycogen to glucose in the liver, as pointed out previously. In depancreatized dogs insulin either did not increase the rate of hepatic glucose production, or the increase was confined to the first twenty-five minutes of the insulin infusion (table 8).

Insulin caused an abrupt increase in the rate of glucose utilization in both depancreatized and normal dogs. This finding is in agreement with the results of previous investigators.[7,21-23] In normal dogs the increase was transient, unless hypoglycemia was prevented by the simultaneous infusion of glucose. Where hypoglycemia was avoided the magnitude of the increase in the rate of glucose disappearance exceeded that found without infusing glucose, and the high rates were maintained throughout the experiment. These experiments support the hypothesis,[24] that the uptake of glucose by the tissues is a function of the concentration of glucose in the surrounding fluid (e.g. interstitial fluid), and thus that the hypoglycemic effect of insulin in normal dogs is self-limiting through *both* the rates of production and utilization of glucose. It is concluded that the hypoglycemia after insulin is mainly due to the accelerated uptake of glucose by the cells of the organism.

SUMMARY

1. The effects of both injection and infusion of insulin on the apparent distribution space and the rates of production and utilization of glucose have been studied in the fasting dog under Nembutal anesthesia, using the validated method of successive tracer injections.

2. There was a delayed increase in the apparent glucose space following the intravenous administration of a hypoglycemic dose of glucagon-free insulin to normal dogs.

3. This change was not seen in fasting depancreatized dogs receiving similar treatment after having been without insulin for forty-eight to sixty hours.

4. The intravenous injection of insulin caused a prompt and appreciable increase in the rate of utilization of glucose in both the normal and depancreatized dogs.

5. In the normal dog insulin administration caused an initial decrease in the rate of hepatic glucose output,

followed by an increase above the pre-insulin rate which at first was insufficient to prevent a fall in blood sugar. This increase in the rate of glucose output was transient or absent in the depancreatized dog following insulin injection.

6. When hypoglycemia caused by the infusion of insulin in the normal dog was prevented by the simultaneous administration of glucose, the rate of hepatic glucose output increased to a lesser extent, or more transiently, while the rate of glucose utilization was markedly increased.

7. Under these circumstances the administered insulin still caused an increase in the apparent glucose space of the normal dog. The working hypothesis that the effect of insulin on the apparent glucose space in the normal but not in the depancreatized dog results from an intracellular breakdown of liver glycogen to substances whose glucose moiety can interchange more rapidly with the circulating extrahepatic glucose is being investigated.

SUMMARIO IN INTERLINGUA

Proratas de Production, Utilisation, e Accumulation de Glucosa e le Apparente Spatio de Distribution de Illo: Effectos de Insulina in Canes, Determinate per Medio de un Provate Methodo a Traciatores

1. Le effectos del injection e etiam del infusion de insulina super le apparente spatio de distribution e super le proratas de production e de utilisation de glucosa esseva studiate in canes in stato jejun sub anesthesia per Nembutal, con le utilisation de un provate methodo de successive injectiones de traciatores.

2. Esseva constatate un retardate augmento in le apparente spatio de glucosa post le administration intravenose de un dose hypoglycemic de insulina sin glucagon a canes normal.

3. Iste alteration non esseva incontrate in dispancreatisate canes in stato jejun quando illos esseva similemente tractate post haber essite sin insulina durante quaranta-octo a sexanta horas.

4. Le injection intravenose de insulina causava un prompte e appreciabile augmento in le proratas del utilisation de glucosa tanto in le canes normal como etiam in le canes que habeva essite dispancreatisate.

5. In canes normal le administration de insulina causava initialmente un reduction del rendimento hepatic de glucosa. Isto esseva sequite de un augmento a nivellos supra illos constatate ante le administration de insulina. Le augmento in le rendimento de glucosa non esseva observate in canes dispancreatisate quando illos recipeva injectiones de insulina.

6. Quando le hypoglycemia causate in canes normal per le infusion de insulina esseva prevenite per le administration simultanee de glucosa, le rendimento hepatic de glucosa cresceva minus marcatemente o plus transientemente, durante que le utilisation de glucosa esseva marcatemente intensificate.

7. Sub iste circumstantias le insulina exogene causava nonobstante un augmento in le apparente spatio de glucosa in canes normal. Es investigate currentemente le hypothese preliminari que le effecto de insulina super le apparente spatio de glucosa in canes normal — un effecto que non occurre in canes dispancreatisate — resulta ab un decomposition intracellular de glycogeno hepatic con le formation de substantias que include un portion de glucosa capace a excambiar se plus rapidemente pro le circulante glucosa extrahepatic.

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On Teaching Diabetes

What Is Needed in the Medical Schools to Improve the Teaching of Diabetes?

We discussed preclinical teaching of diabetes and the vertical versus the horizontal approach. Someone asked what these symbols might mean. In the first an attempt is made to co-ordinate the special interests of each department into teaching at every level. In the second each discipline is taught separately and its application to a disease is made apparent during the teaching of the subject at hand. The problem of utilizing clinical faculty in the teaching of basic sciences and the problem of utilizing basic science personnel in clinical teaching were discussed, and it was felt that the latter was the more important. There was discussion concerning whether integration should be encouraged by small group teaching or whether this should be applied to large classes. It was felt that this depended upon the availability of faculty in any particular institution.

We then directed our attention to the use of the teaching clinic. It was stressed that the teaching clinic (in this case the diabetes clinic) frequently deteriorates to a service function and that exposure to or experience with patients is not actually training. Adequate supervision is required at both the student and house staff level. With an indigent patient population the students and house staff would get a distorted view of the practice of diabetes. Wherever possible the student and house staff should have an opportunity to treat private patients and to learn the mechanisms of taking care of private patients with diabetes. The problem of continuity in the entire training program was stressed. It was felt that the block system, in which a student and house staff are in the clinic for only six to eight weeks, is not the preferable method. It is preferable to have a student in the clinic one afternoon or one morning a week for a period of several months, in order to learn something of the natural history of diabetes. It was felt, also, that for continuity it was essential to utilize the services of a nurse, social worker, dietitian, and perhaps also a psychiatrist and ophthalmologist wherever possible. A large number of patients is not essential for proper training of students and house staff. Exposure to a few patients with adequate supervision is preferable to exposure to a large number of patients with poor supervision.

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Secondary Failure of Tolbutamide in the Normal Rat

Correlation Between Beta-cell Granulation and Blood Sugar Response

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Tolbutamide is usually not an effective hypoglycemic agent when given to severe or juvenile diabetics. On the other hand, its use in controlling elevated blood sugar levels in adults with mild diabetes or in diabetes which appears late in life has been gratifying. With prolonged use of this drug, however, several reports have appeared in the literature pertaining to secondary failure of response to tolbutamide treatment.[1-3] If a patient shows a hypoglycemic response for one month or more and then the drug loses its effectiveness, this is called secondary failure. The exact cause of this failure is not clear but it was thought earlier that it was because the beta cells of the islets had become degranulated and consequently were unable to release insulin.

In short-term studies in experimental animals, the degranulation of beta cells following sulfonylurea treatment has been noted by many investigators.[4-6] With chronic administration to subdiabetic rats, however, Lazarow and Treibergs[7] found no hypoglycemic response to tolbutamide. In regard to beta-cell granulation, several workers[6,8,9] have reported degranulation after feeding tolbutamide for several weeks. However, Lundbaek and Nielsen[10] noted that beta-cell granulation was normal in rats injected with tolbutamide for forty-five days.

The present study was undertaken in order to compare the degree of granulation of beta cells with blood sugar levels in tolbutamide-fed normal rats. It was of particular interest to try to determine whether secondary failure of tolbutamide occurs in the normal adult animal.

MATERIAL AND METHODS

Mature female rats of the Long-Evans strain were fed for one week on a synthetic diet consisting of the following: casein (purified), 350 gm.; sucrose, 600 gm.; corn starch, 600 gm.; yeast (Pabst), 200 gm.; salt mixture (Hubbel, Mendel & Wakeman), 50 gm.; hydrogenated cottonseed oil, 100 gm.; cottonseed oil, 100 gm.; choline hydrochloride, 400 mg.; haliver oil, 4 ml.

Then 0.5 gm. of sodium tolbutamide was added to each 100 gm. of the synthetic diet and this was fed for the following six weeks. Nonfasting blood sugar levels were determined by the Nelson-Somogyi method at weekly intervals and the averages on the graphs represent values for at least ten rats. Eight to ten animals were killed each week for pancreatic studies. The pancreas was fixed in Bouin's fluid twelve to eighteen hours and embedded in paraffin during the next six hours. Sections six microns thick were stained with Gomori's aldehyde-fuchsin technic after oxidation with potassium permanganate.

An estimation of beta-cell granulation was made without the identity of the material being known to the observer. The granulation was rated from 0 to 4 plus, the latter representing maximum granulation.

The variance shown on the graphs for blood sugar levels is an estimate of variation and shown as the standard error of the mean.

RESULTS

After the rats had been fed the synthetic diet for one week the average nonfasting blood sugar level was just below 100 mg. per 100 ml. One week after tolbutamide had been added to the diet, however, the blood sugar level was 47 mg. per 100 ml. (figure 1). With additional feeding of the tolbutamide diet there was a gradual increase in blood sugar levels so that at six weeks it was near its initial control level, i.e., 92 mg. per 100 ml.

The marked decrease in blood sugar noted after feeding tolbutamide for one week cannot be attributed entirely to the action of this drug. It has been found that the tolbutamide diet causes a mild anorexia. Rats during the control period ate on the average between eleven and twelve grams of the synthetic diet daily. When tolbuta-

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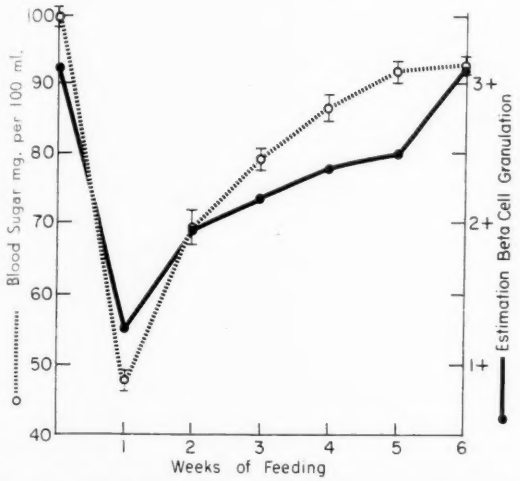

FIG. 1. Effect of feeding tolbutamide on blood sugar levels and beta-cell granulation.

mide was added there was an immediate reduction in food intake which averaged approximately 1 gm. less than that eaten during the control period. In fact, the tolbutamide-fed animals ate approximately 1 gm. less during the first three to four weeks and then returned to the level of the initial feeding period. Reduction in food intake led to weight loss, most marked during the first week the animals were given tolbutamide. By the sixth week of feeding body weights were near the original control level.

Of course, the granulation of the pancreatic beta cells, and a comparison of the degree of granulation with blood sugar levels, is of interest in this study. Marked degranulation of the beta cells was present after tolbutamide had been fed for one week (figure 1). With continuous feeding of tolbutamide there was a rapid recovery of the granulation at two weeks and at the six-week period the granulation was similar to that found in the control animals before tolbutamide was fed. Photomicrographs are shown of islets from animals fed the tolbutamide diet for 0, 1, 3 and 6 weeks (figure 2).

Even though the identity of the pancreatic tissue was unknown to the observer who estimated the degree of granulation of the beta cells, this method of study may be open to criticism. The same tissue was studied using a densitometer for determining the density of granules in the beta cells.

Since the apparatus used was rather crude, the data for the densitometer studies are not included, but, in general, the results were similar to those obtained by visual observation of the islets.

DISCUSSION

The present study demonstrated clearly that secondary failure to tolbutamide occurs in the normal rat. When a constant amount of tolbutamide is added to the rat's diet, marked hypoglycemia is present the first week but the blood sugar level rises somewhat thereafter until by the sixth week it is nearly back to normal levels. Part of the depression in blood sugar levels during the first three or four weeks of the experiment may be because the animals were eating somewhat less food at this time.

It is of particular interest to note the similarity of the blood sugar curve and the amount of beta-cell granulation when the two are plotted on the same graph. The blood sugar levels are the lowest when the most degranulation has occurred. Likewise, as the granulation increases, so do the blood sugar levels.

The secondary failure of tolbutamide, as demonstrated in the present study, occurred much more rapidly than was expected. Because Long-Evans strain of the rats react in the manner described, need not imply that all strains of rats or other animals would respond in the same way. The reason the rats used in the present study were able to adapt themselves to prevent the action of tolbutamide is not known. Some diabetic human beings who respond to tolbutamide therapy initially display secondary failure in a certain number of cases,[1-3] but many have been treated for several years without developing tolerance to this drug with loss of hypoglycemic effect.

SUMMARY

Normal rats of the Long-Evans strain were fed a synthetic diet containing 0.5 per cent sodium tolbutamide. Blood sugar levels and islet beta-cell granulation were determined at weekly intervals for a period of six weeks. The blood sugar level was lowest and degranulation of the beta cells most marked after one week of the diet. During the following five weeks the blood sugar levels and beta-cell granulation gradually increased until they approached values found during the pretreatment period. The rat, thus, exhibits secondary failure to tolbutamide. It appears that since the beta cells no longer show degranulation and blood sugar levels have returned to normal levels that the beta cells, for some unknown reason, have become less responsive to tolbutamide.

SUMMARIO IN INTERLINGUA

Disfallimento de Tolbutamida, Como Occurrentia Secundari in le Ratto Normal: Correlation Inter le Granulation del Cellulas Beta e le Responsa del Sucro Sanguinee

Rattos normal del racia Long-Evans recipeva un dieta

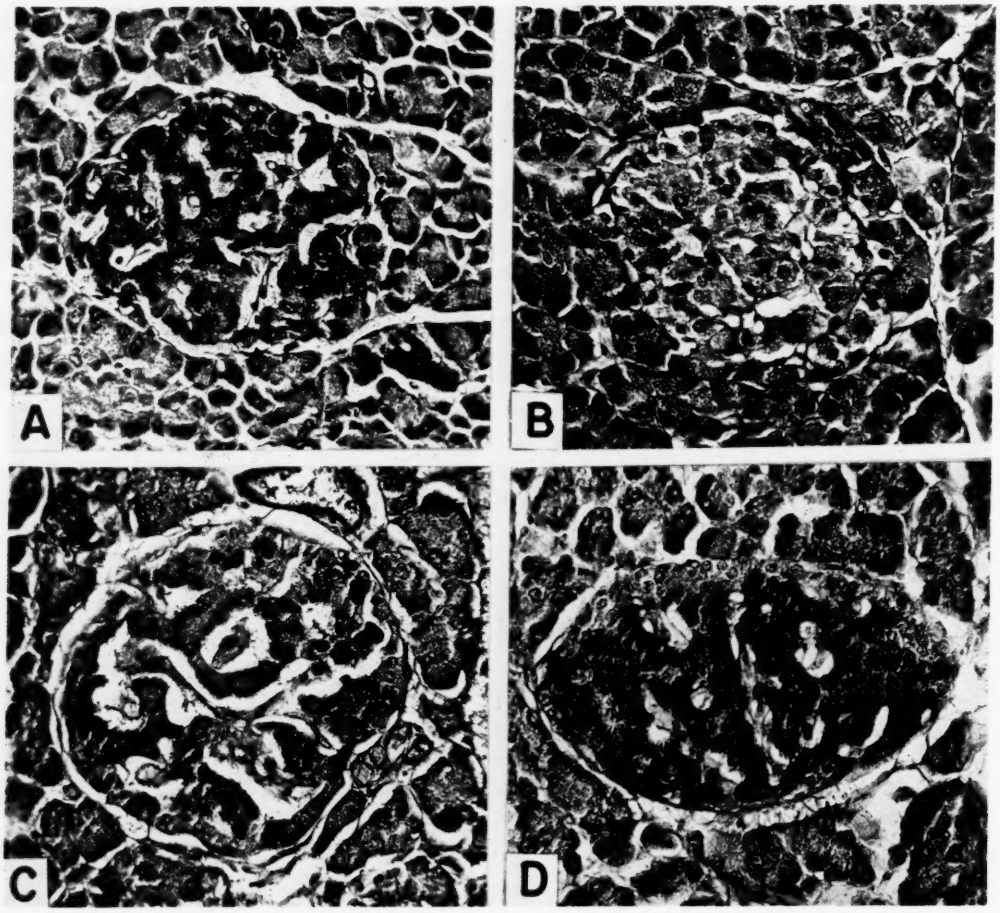

FIG. 2. Effect of feeding a diet containing 0.5 per cent tolbutamide on the beta cells of pancreatic islets. A. Control rat. Estimation of beta cell granulation 3.00. B. Islet from rat fed tolbutamide for one week. Estimation of beta cell granulation 1.25. C. Islet from rat fed tolbutamide for three weeks. Estimation of beta cell granulation 2.25. D. Islet from rat fed tolbutamide for six weeks. Estimation of beta cell granulation 3.25.

synthetic continente 0,5 pro cento de tolbutamida a natrium. Le nivellos del sucro sanguinee e le granulation del cellulas beta in le insulas esseva determinate a intervallos septimanal durante un periodo de sex septimanas. Le nivello del sucro sanguinee esseva le plus basse e le disgranulation del cellulas beta le plus marcate post un septimana del dieta. Durante le sequente cinque septimanas le nivellos del sucro sanguinee e le granulation del cellulas beta se re-accentuava gradualmente usque illos esseva proxime al valores constatate durante le periodo ante le tractamento. Assi le ratto exhibi, in responsa a tolbutamida, un disfallimento secundari. Il pare que-viste que le cellulas beta cessa exhibir disgranulation e viste que le nivellos del sucro sanguinee ha retornate a nivellos normal — le cellulas beta (pro le un o le altere indeterminate ration) ha devenite minus responsive a tolbutamida.

ACKNOWLEDGMENT

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Hypoglycemia Induced by Galactose

Galactosemia, an hereditary disorder which appears in infants, is evidenced by an increased galactose level in the blood (as shown by increased total reducing sugar levels) and by poor growth. Because of an accumulation of galactose-1-phosphate, it is believed that galactosemia is due to a deficiency of a specific enzyme (galactose-1-phosphate uridyl transferase) which allows further metabolism of this compound.

Recently, T. L. Dormandy, D. Leak and M. Grant (Lancet 2:269, 1959) have studied the effect of galactose on the level of glucose in the blood. Using the glucose oxidase method for the determination of blood sugar, it was possible for these investigators to determine changes in the glucose content of the blood when there were large quantities of galactose or other reducing sugars present.

In studying six volunteers, each of whom was given an oral galactose tolerance test after taking 50 gm. of galactose, transient slight falls in blood glucose were noted in only three of the six individuals. After receiving 100 gm. of galactose by mouth, four of the six volunteers showed only a slight decrease in blood glucose during the first hour following the ingestion of the sugar. However, when a high blood galactose level was obtained by giving the galactose intravenously, five of the six volunteers showed depressions in the blood glucose levels within a few minutes after the galactose had reached its peak value (total reducing sugar about 325 mg. per 100 ml.). Under these conditions the maximal decrease in the blood glucose amounted to approximately 30 mg. per 100 ml. and was persistent for about ninety minutes.

Thus it appears that with this group of volunteers (apparently adults) the elevation of the total blood sugar produced by giving galactose intravenously caused a fall in the blood glucose levels.

Since lactose (hydrolyzed to galactose) may be less efficiently utilized in the neonatal period than it is in the adult, these investigators carried out preliminary studies on six prematures and three full-term infants. . . . They studied the galactose and glucose levels while the infants were maintained on their ordinary feeding schedules, and in this manner employed a "rough and ready" galactose and glucose tolerance test. In the case of seven of the nine infants, there was an increase in the blood glucose levels in the hour and a half following the feedings.

However, in one premature infant and one premature and jaundiced infant there were marked decreases in the blood glucose along with the elevation of the total blood sugar during the early period following the oral feeding, the fasting blood sugar was 47 gm. per 100 ml. and it fell to a minimum value of 15 mg. per 100 ml. forty-five minutes after the feeding. By the third day of life the tolerance of galactose had increased so that, in spite of a minimum blood glucose value of 19 mg. per 100 ml. reached fifteen minutes after feeding, the blood glucose returned to the fasting level within one hour after feeding, remaining at approximately the same level for the next hour.

The tests were repeated on the tenth and twentieth days and, in both cases, the blood glucose increased greatly after feeding with only small increases in the total reducing sugar levels. (*Continued on page 319.*)

The Effect of a Fish-oil Fraction on Plasma Lipids

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Since the original report from this laboratory in 1952[1] many investigators have confirmed that the substitution in the diet of vegetable fats containing large amounts of linoleic acid (e.g., corn, cottonseed, safflower, soy oil) for fats containing predominantly saturated fatty acids (e.g., fat from meat, eggs, and dairy products) will result in a marked fall in plasma lipids in most human subjects. It has further been demonstrated that the addition of sufficient amounts of polyunsaturated fats to (a) fat-free diets or (b) diets containing significant amounts of saturated fats will result in a significant fall in the level of plasma lipids.[2] The amount of vegetable fat required under the latter set of conditions is of considerable magnitude and from a clinical standpoint is unfeasible because of the increase in dietary calories.

Recently, Ahrens and his associates[3] reported that menhaden oil, a fish oil with an iodine value in excess of 150, had at least as great an effect upon plasma lipids as an equal amount of vegetable oil containing more than 50 per cent of linoleic acid.

On the basis of existing information there is some reason to believe that the efficacy of a fat in lowering plasma lipids may be in a measure proportional to the total number of double bonds present in its fatty acids. Conceivably, a highly unsaturated fat containing fatty acids of proper molecular configuration would be capable of lowering plasma lipids when added in relatively small quantities to a diet containing considerable quantities of saturated fat. Accordingly, the ethyl esters of menhaden oil were fractionated by appropriate methods with the yield of the fish oil fraction shown in figure 1. The gas-liquid chromatographic pattern of menhaden oil fatty acids is shown at the top of this figure, the menhaden ethyl ester fraction in the center, and a standard fatty acid mixture is shown at the bottom for orientation purposes. It is apparent that the fractionated material contains very much larger amounts of long-chain highly unsaturated material than the original menhaden oil. The iodine value of this material was slightly over 300. It contained 24 per cent of a five double bond C_{20} acid and 35 per cent of a six double bond C_{22} acid. This material was given to patients maintained under quantitatively constant dietary conditions in the metabolic ward.

The three women whose studies are illustrated in figures 2, 3, and 4 were mild diabetics, with adult onset of their disease and no evidence of ketosis at any time. They were forty-five, seventy, and fifty-five years of age respectively. SDEB (figure 2) had no hypoglycemic therapy prior to her metabolic ward admission, except for a diet to which she adhered rather poorly. She had 0—2+ glycosuria and an average fasting blood sugar of 200 mg. per 100 ml. LSOU (figure 3) was known to have had diabetes for one year, and had received a 1,200 calorie "diabetic" diet, to which she adhered rather poorly, and reserpine for mild hypertension, prior to this admission. She also had a fasting blood glucose which averaged approximately 200 mg. per 100 ml. before admission. Her urine sugar tests were usually negative. MMAR (figure 4) had diabetes of eleven years' known duration and had been maintained on 20 to 30 units NPH insulin with no glycosuria and a fasting blood glucose of 120-170 mg. per 100 ml. before her metabolic ward admission. She was maintained on 25 units NPH insulin daily while on the metabolic ward. The other two patients received no hypoglycemic medication. Diabetic control of all three patients was excellent during their stay on the metabolic ward.

In figure 2 a study is shown in which the fish oil fraction, designated as "Escambia Oil," was substituted in calorically equal amounts for 80 gm. of butter fat. There was a prompt decrease in the level of total lipids and of total cholesterol.

The inclusion of this amount of the Escambia Oil resulted in a formula which was quite objectionable in taste. Accordingly, the material was prepared in soft gelatin capsules, each containing one gram of the ethyl esters, and the material was administered in this form to a mildly diabetic patient who was maintained on a quantitatively constant formula in which all of the fat initially was derived from butter (figure 3). Then 12 gm. of

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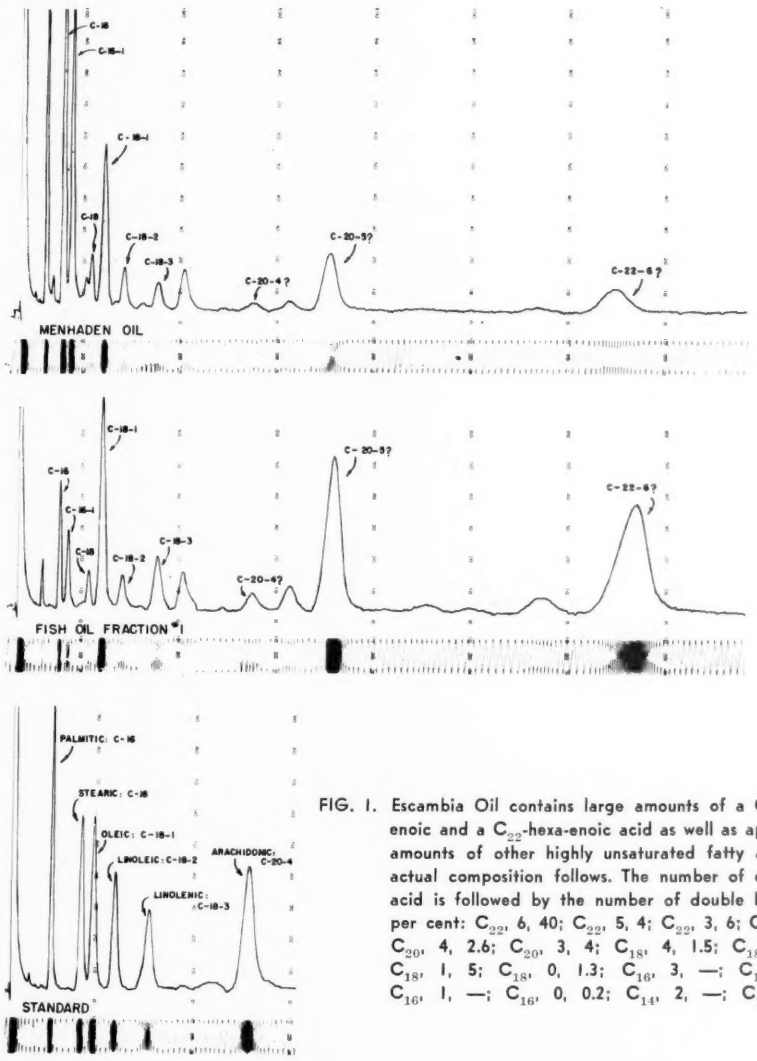


FIG. 1. Escambia Oil contains large amounts of a C_{20}-penta-enoic and a C_{22}-hexa-enoic acid as well as appreciable amounts of other highly unsaturated fatty acids. The actual composition follows. The number of carbons in acid is followed by the number of double bonds and per cent: C_{22}, 6, 40; C_{22}, 5, 4; C_{22}, 3, 6; C_{20}, 5, 30; C_{20}, 4, 2.6; C_{20}, 3, 4; C_{18}, 4, 1.5; C_{18}, 2, 0.5; C_{18}, 1, 5; C_{18}, 0, 1.3; C_{16}, 3, —; C_{16}, 2, —; C_{16}, 1, —; C_{16}, 0, 0.2; C_{14}, 2, —; C_{14}, 0, —.

Escambia was added to the butter fat. Subsequently, 20 gm. of Escambia Oil replaced 20 gm. of butter fat. A significant decrease in the level of plasma lipids resulted.

In figure 4 another study is shown, in which initially 8 gm. of Escambia Oil were added to 80 gm. of butter fat with a prompt fall in plasma lipids. The oil was subsequently increased to 16 gm. with no additional effect.

At the time of submission of this report the material has been administered to four "essential" familial hypercholesterolemic persons and one "essential" hyperglyceridemic individual, all on an ambulatory basis. In none of these patients has there been a response in terms of significant and maintained fall in plasma lipids. In the immediate future the material will be administered to a variety of ambulatory patients with elevated lipids, including diabetic patients with retinal and renal involvement. With the exception of mild diarrhea in one patient, who had previously shown such intolerance to other fats, and abdominal distress in one of the patients who also had biliary calculi, no untoward effects have been noted.

SUMMARY

The present study indicates that a fish oil fraction con-

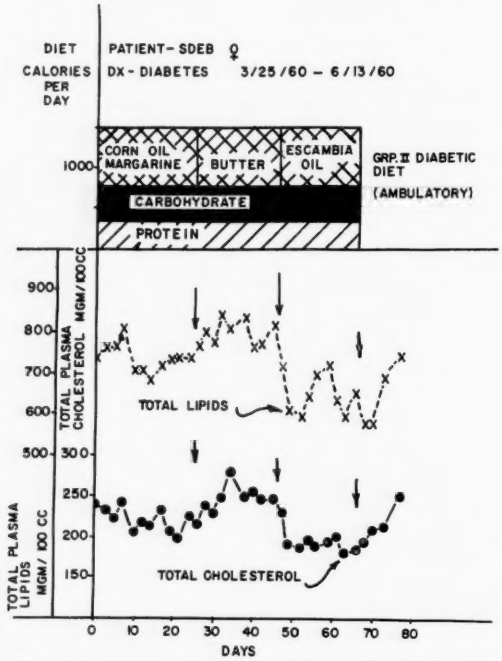


FIG. 2. Replacement of 80 gm. of butter fat by 80 gm. of Escambia Oil was associated with a significant fall in plasma lipids.

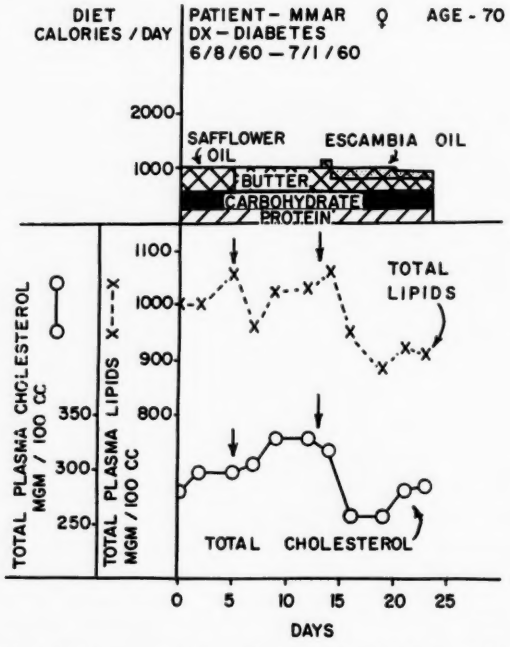
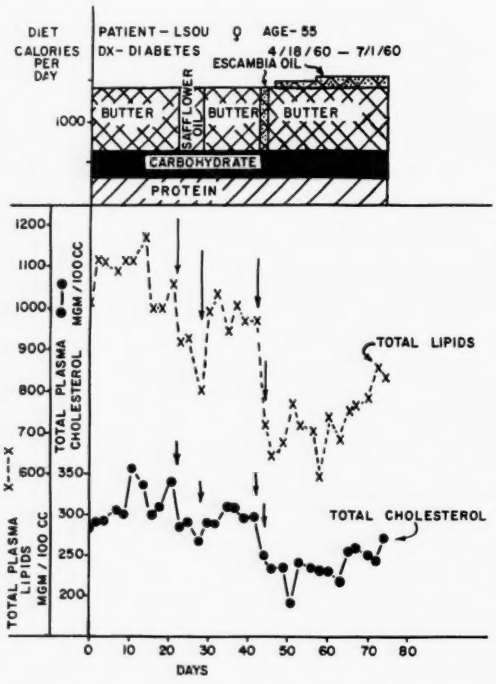


FIG. 3. Replacement of a portion of butter fat by Escambia Oil resulted in a decrease in plasma lipids.



taining large amounts of long-chain, polyunsaturated fatty acids which are not "essential" in the usual sense of the word[3] will produce a significant decrease in the level of plasma lipids in some individuals in the presence of a relatively large amount of dietary saturated fat. The amounts required per day to produce this effect vary from 8 to 30 gm. Effects of this magnitude have not been observed in this laboratory when equal amounts of ethyl linoleate or vegetable fats have been added to diets containing an abundance of saturated fat. It seems probable that the efficacy of this material is in a measure proportional to its unsaturation.

Work designed to produce fractions containing much larger proportions of the penta- and hexa-enoic acids is under way. Such fractions may conceivably produce hypolipidemic effects in much smaller dosage. It seems unlikely that such a preparation will take the place of a dietary modification, but it may possibly be useful as a dietary supplement.

FIG. 4. Addition of 8 to 16 gm. of Escambia Oil to a formula containing 90 gm. of butter fat produced a major fall in plasma lipids.

SUMMARIO IN INTERLINGUA

Le Effecto de un Fraction de Oleo de Pisce Super le Lipidos del Plasma

Le presente studio indica que un fraction de oleo de pisce que contine grande quantitates de poly-nonsaturate acidos grasse a catena longe, non "essential" in le senso ordinari del parola, produce un declino significative del nivello de lipidos de plasma in certe subjectos in le presentia de relativemente grande quantitates de grassia saturate in le dieta. Le quantitates requirite pro producer iste effecto varia ab 8 ad 30 g per die. Effectos de iste magnitude non esseva observate a iste laboratorio quando simile quantitates de linoleato ethylic o de grassias vegetal esseva addite a dietas continente un abundantia de grassia saturate. Il pare probabile que le efficacia de iste material es, in un certe mesura, proportional a su non-saturation.

Es in progresso travalios visante a producer fractiones continente multo plus grande proportiones del acidos penta- e hexa-enoic. Il pare plausibile supponer que tal fractiones es possibilemente capace a producer effectos hypolipidemic post lor administration in quantitates multo minus considerabile. Il non es probabile que un tal preparato pote prender le placia de un modification dietari, sed illo va possibilemente provar se utile como supplemento dietari.

ACKNOWLEDGMENT

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The Escambia Oil was supplied by Dr. Robert Hodgkinson of Parke, Davis and Company.

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Hypoglycemia Induced by Galactose

(*Continued from page 315.*)

Similar trends were noted in the second premature infant who was jaundiced, and in this case, while the fasting blood glucose was 32 mg. per 100 ml. on the third day of life, it fell to a minimum value of 13 mg. per 100 ml. thirty minutes after feeding and attained a fasting value about thirty minutes later. During this time, the total blood sugar (representing mainly galactose) steadily increased, reaching a maximum value at thirty minutes (90 mg. per 100 ml.). On the fourth day of life, similar levels of blood glucose were noted, but by the ninth day there was an increase in the blood glucose level after ingesting food and the galactose was at its maximum, only 20 mg. per 100 ml.

While none of these individuals showed definite clinical signs of hypoglycemia, the possibility could not be excluded that hypoglycemia produced by galactose could be a clinical as well as a biochemical feature in the neonatal period. The chemical detection of such a hypoglycemia would require the use of the glucose oxidase method.

The means by which the increased blood galactose influences the blood glucose is not known. The possibility exists that the blood galactose stimulates the pancreas directly to produce increased amounts of insulin. Also, the release of glucose by the liver may be influenced by the elevated blood galactose. These investigators suggest that the metabolic regulatory system responsible for maintenance of the blood glucose within its normal narrow limits apparently cannot readily differentiate between glucose and galactose. Thus a sharp rise in the blood galactose will cause a decrease in the blood glucose level.

From *Nutrition Reviews*, Vol. 18, No. 2, pp. 38-39, February 1960.

Multiple Keloids Formed at the Sites of Insulin Injections

A Case Report

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The insulin injections which diabetic patients have received in astronomical numbers throughout the world have heretofore not been reported to produce keloids at the site of injection. This is surprising since keloids are usually secondary to physical, chemical or thermal trauma in susceptible individuals, notably in Negroes. According to various observers the incidence of keloids in white and Negro individuals is in the ratio of 1:9 to 1:16.

The following case is of interest because it is the first report to our knowledge of multiple keloids formed at the sites of insulin injections.

CASE HISTORY

A. H., a forty-five-year-old colored female, was first seen in the Mt. Sinai Hospital dermatology clinic in 1945 with a complaint of itching, tender scars on the anterior chest of two years' duration. She stated that multiple scars appeared on areas of irritation and scratching. The scar tendency had its onset following marriage and her first pregnancy.

Physical examination at that time was essentially normal with the exception of the skin and subcutaneous tissue. On the anterior chest an infected keloid two inches by four inches in diameter was noted with a sinus draining purulent material. Keloid scars were also present on the right ear lobe, the abdomen, and legs.

The patient gave no history of other trauma, and there was no familial history of keloids in her parents and eight siblings. Five children ranging in age from four to twenty were free of keloids at that time and have remained so to date. In 1945 patient was admitted to the hospital for the purpose of excision of the keloid, but was discharged two days later and referred to radiotherapy, without any surgical intervention.

The patient developed diabetes mellitus in 1948 and was controlled with diet and daily injections of Protamine Zinc Insulin in gradually increasing amounts. In 1956 keloid scars began to appear at the sites of insulin injections. The lesions began as small papules, with itching and subsequent scratching. Hard nodules surrounding the points of injections then formed (figure 1). The time span of this cycle was two to three weeks. As the lesions became more numerous the patient was reluctant to take her insulin at daily intervals. As a consequence her diabetes became poorly controlled.

It was felt that the patient's daughter might have failed to use sterile technic in giving the injections, but this was excluded by actual observation in the patient's home.

FIG. 1. Left arm showing multiple keloids at sites of insulin injections.

Subsequently, when the oral hypoglycemic agents came into use, the patient was placed on tolbutamide. This drug failed to control her diabetes. The patient resumed daily injections of Protamine Zinc Insulin, and these have continued to be followed by keloid nodules surrounding the sites of some of the injections. In 1959 patient was given ten subcutaneous injections of 0.5 cc. vitamin B-12 (25 μg.) into the right thigh without the formation of a keloid nodule.

A biopsy of a nodule on the right shoulder was performed in December 1959. The microscopic examination showed typical keloid formation without any evidence of foreign body reaction.

From the Department of Medicine and the Diabetic Clinic of the Mount Sinai Hospital, New York, New York.

COMMENT

All the typical features of keloids are found in this patient; the formation of a papule and deep seated nodule, with pruritis and traumatization by scratching are well documented. The pathologic examination of a biopsied nodule failed to reveal the presence of any foreign body reaction to zinc in the lesion. There was no immediate type of reaction locally, after the injection, which might suggest a local sensitivity to insulin. There was no evidence of infections at the sites of injections, and the patient was observed very closely to ascertain that there was no break in the sterile technic.

It is also apparent that since insulin was given daily the nodular reaction did not follow each injection. The ten injections were actually placebo injections (B-12) to eliminate the possibility that the keloids were formed by the needle punctures. No new keloids were formed at their sites.

New keloids presently appear at the sites of insulin injections at the same rate as when first noted in 1956.

We can only speculate that some local factor is present or had arisen in this patient that caused the nodular reactions after taking Protamine Zinc Insulin injections daily for eight years and then only after some of the injections.

SUMMARY

We have observed a patient with a history of multiple keloids of seventeen years' duration who began to form keloids at the sites of Protamine Zinc Insulin injections after taking insulin for eight years.

SUMMARIO IN INTERLINGUA

Cheloides Multiple, Formate al Sito de Injectiones de Insulina

Nos ha observate un patiente con un anamnese de multiple cheloides de un duration de dece-septe annos. Le formation del cheloides comenciava al sitos de injection de insulina a zinc e protamina post octo annos de uso del droga.

EDITORIAL

PHENFORMIN AND KETOACIDOSIS

In this issue of DIABETES appears an article by Walker and Hannah in which is recorded their experience with phenformin (DBI). Although their results were in general similar to those reported by others, they were unusual in the relative frequency with which ketoacidosis was encountered. Ketonuria was noted in thirty-four or 29.1 per cent of 117 patients and in thirteen cases an "alkali reserve" of less than 20 mEq./L. was recorded. Moreover, there was one death in a young man who was admitted to the hospital in coma; although the blood sugar was not greatly elevated, 208 mg. per 100 ml., the alkali reserve was reduced to 3.1 mEq./L. A second fatality occurred in a man aged thirty-seven years who discontinued phenformin when nausea appeared with a febrile illness. Five days later he died shortly after admission to the hospital. Postmortem examination revealed lobar pneumonia with abscess formation.

The report of Walker and Hannah is disquieting and prompts consideration as to the cause of the high incidence of ketonuria and ketonemia. The occurrence of ketonuria in certain subjects receiving biguanides is well recognized but its frequency is poorly documented. In most articles reporting the use of phenformin in large groups of patients, ketonuria is given only brief mention.[1,2] It would appear from this that symptomatic ketoacidosis has not been a significant problem in the hands of most clinicians using phenformin. Among possible reasons for the discrepancy between the experience of Walker and Hannah and that of others, the following come to mind: (a) In certain clinical trials the urine may not be tested routinely for acetone and so the true incidence of ketonuria may not be apparent; (b) the amount of carbohydrate in the diet, the frequency with which insulin is given concomitantly and the degree of control of diabetes sought may vary considerably from one series of patients to another; and (c) the criteria for the selection of patients may differ.

Among the 117 patients studied by Walker and Hannah there were seventeen who were classified as having "juvenile stable" diabetes. Six of these were treated with success by the giving of phenformin alone. One

will await with interest the results of prolonged observation of these patients. Early in the course of childhood diabetes remissions are common and during such periods the production and release of endogenous insulin seem to be decreased relatively little and in some instances to approach normal. Almost invariably the time comes when these patients become truly dependent upon injected insulin and are characteristically ketosis-prone. In this group, even during stages of remission, convenience in treatment must be given a secondary role and therapy planned so that the maximum protection afforded by insulin may be provided.

In thirty-seven "juvenile unstable" cases Walker and Hannah had "temporary success" in some but had the uncomfortable experience of finding that "good control for long periods may be terminated suddenly and unexpectedly by ketoacidosis." They conclude quite rightly that phenformin should not be used alone in such patients and give as an added reason the fact that the majority of young patients on phenformin alone tend to lose or fail to gain weight.

Although uncertainty still exists as to the mechanism by which biguanides lower the blood glucose, there is much evidence to suggest that these chemical agents inhibit cellular oxidative reactions and bring about increase in the breakdown of glucose by means of anaerobic glycolysis. Their use is followed by an accumulation of intermediate metabolites of the Krebs cycle, a decrease in the rate of disappearance of pyruvate, lactate and citrate and a decrease in the oxidation of glucose, acetate and succinate.[3] One would not expect phenformin or its analogues to prevent or overcome ketoacidosis in a ketosis-prone diabetic subject and, indeed, there is no evidence to suggest that these compounds exert an antiketogenic effect. In fact, Walker and Hannah bring up the possibility that the drug may antagonize the antiketotic effect of insulin. At any rate, these biguanides may allow ketonemia and ketonuria to develop under conditions in which there is inadequate glucose oxidation at the cellular level. The ketonuria may, but by no means always, represent the relatively benign normoglycemic ketosis of hunger or starvation which usually responds to increase in the amount of carbohydrate in the diet or lowering of the dose of the biguanide or both.[4,5] It may indicate, at least in part, true diabetic ketoacidosis which can be overcome only by the giving of insulin in adequate dosage. This important point is stressed by Walker and Hannah whose clinical investigation included patients who later, on the basis of experience, they concluded were not proper subjects for phenformin therapy.

The report of Walker and Hannah deserves special comment because it calls attention to the way in which phenformin should or should not be used, if it is the decision of the physician to try this drug. Phenformin lowers the blood glucose in certain older patients with maturity-onset, stable diabetes. Individuals in this category presumably retain to a considerable degree the ability to produce and release insulin. An entirely different situation prevails in children and young adults with growth-onset diabetes and older persons with unstable diabetes. These patients usually develop ketosis easily and represent a group in which endogenous insulin production presumably is small, or in some instances, probably lacking. Such individuals require injections of insulin daily for health and, indeed, for survival. In them insulin should form the basis and mainstay of treatment and if the physician desires to try phenformin it should be used only as a supplement to insulin. In this class of patients INSULIN may be supplemented by PHENFORMIN but *not* PHENFORMIN with INSULIN!

REFERENCES

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[3] Duncan, L. J. P., and Baird, J. D.: Compounds administered orally in the treatment of diabetes mellitus. Pharm. Reviews: *12*:91, 1960.

[4] Creutzfeldt, W., and Söling, H. D.: Toxizität und nebenwirkungen der Guanidine und Biguanide. In Bertram, F., and Michael, G.: Internationales Biguanid-Symposium. Stuttgart, George Thieme, 1960, p. 37.

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ALEXANDER MARBLE, M.D.
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BOOK REVIEWS

METAL-BINDING IN MEDICINE. *Edited by Marvin J. Seven, M.D. $13.75, pp. 400, J. B. Lippincott, Philadelphia, 1960.*

The present importance and understanding of the role of trace elements in medicine is the result of several parallel developments. The identification of metal ions as cofactors for the activity of many enzymes has clarified the nutritional need for certain elements and at the same time has provided the best understood cases of the mechanism of enzyme action. During the same period, the study of the physical and organic

chemistry of chelation has led to illuminating theoretical treatments and to the availability of chelating agents with special properties such as EDTA. The combination of these developments has resulted in many applications to medical problems. *Metal-Binding in Medicine* is a volume of contributions based on a symposium on this subject. The six sections into which the book is divided cover the range of the relationships of metal ions from the mechanism of enzyme action to the control by chelation of their concentration in body fluids and of their entrance and exit from the body. The subjects treated include iron metabolism, copper metabolism, the use of chelates in aiding the excretion of undesirable metals, and the manipulation of plasma calcium concentration with EDTA. Taken together the chapters on applications provide a surprisingly systematic account of the subject for this type of volume. An effort has been made to include a background in the principles of chelation. Valuable as these chapters are they do not prepare the general reader for much of the material that follows. While an elementary presentation would have been out of place in the symposium it might have made the book more useful to those primarily interested in the applications of chelation to medicine.

PUBLICATIONS BY DR. HANS-WALTHER LARSEN: 1. DIABETIC RETINOPATHY, *Copenhagen, 1960.* 2. ATLAS OF DIABETIC RETINOPATHY: A Photographic Study, *Reports of the Steno Memorial Hospital and the Nordisk Insulinlaboratorium, Vol. VIII, Copenhagen, 1959.* 3. X-RAY THERAPY IN PROLIFERATIVE DIABETIC RETINOPATHY. *Acta Ophthalmologica, Vol. 37, 1959.*

These publications of Dr. Larsen present an excellent review of the history and available literature on diabetic retinopathy. In addition, and most important, they provide a detailed description of the ophthalmoscopic picture. This is based on the careful study of a large number of patients seen at the Steno Memorial Hospital.

Although the first publication listed above presents an excellent review for all interested in the vascular complications of diabetes far and away the most important part of the study is the *Atlas of Diabetic Retinopathy: A Photographitc Study.* In this monograph is presented a most superb set of over one hundred fundus photographs demonstrating in detail various stages of the disease process, its progression and remission. This atlas should be made generally available not only to ophthalmologists but to all physicians interested in ophthalmoscopic examinations. The photographs are of such excellent quality and demonstrate the ophthalmoscopic picture so exactly, that they should come to replace fundus drawings in all teaching collections.

The third publication on X-ray therapy merely confirms the failure of this as well as the other forms of therapy in proliferative diabetic retinopathy.

PRINCIPLES OF HUMAN GENETICS. *By Curt Stern, $9.50, pp. 753, W. H. Freeman and Co., San Francisco 4, California, 1960.*

This reviewer remembers being greatly impressed, when he was a graduate student, by the beauty and clarity of Curt Stern's demonstration in Drosophila melanogaster that chromosomes exchange parts when crossing over occurs, and by his brilliant demonstration that chromosomes of somatic cells may also cross over. Stern has continued to do brilliant experiments with Drosophila and to explain them with great clarity. For almost thirty years I have had the pleasure of reading successive reports by Stern explaining with unusual clearness his fundamental experiments.

It was not surprising, therefore, to find that the first edition of *Principles of Human Genetics,* which appeared in 1949, was a brilliant exposition of the elements of human genetics. It was, as he has said, the result of his attempt to learn the technics of what was for him a new field of genetics. The success of that first edition is ample comment on how well he learned and explained the subject.

The second edition, which appeared in 1960, was necessitated by the rapid advances occurring in human genetics. This edition is essentially a new book. All chapters have been rewritten and six new chapters have been added. These chapters are: "The Detection of Linkage," "Variations in the Expression of Genes," "Genetic Hazards of Radiation," two additional chapters on "Heredity and Environment" and "Selection and Genetic Polymorphism."

The material is current, the interpretations are modern, and often original, the presentation is clear and written with warmth and modesty. Stern is scrupulously fair in presenting controversial material; too much so in certain instances, in my opinion, but these are such minor and such technical points that they are best not discussed here.

No prior knowledge of genetics is required to read this book, hence any physician should be able to read it (not as he would a novel, to be sure). Any one who does, and who works the helpful problems at the ends of the chapters, will have a firm foundation in human genetics and will be able to read with understanding the genetic literature pertinent to his interests.

Although Dr. Stern is not a physician, he has not neglected the more clinical aspects of the subject. The chapters on "Genetic Counseling," "The Genetic Hazards of Radiation," "Heredity and Environment," and "Aspects of Medical Genetics" are directly pertinent. There is, in addition, much of clinical application in most of the other chapters.

Excellent bibliographies appear at the end of each of the chapters to permit those who are interested to pursue a given topic in greater detail.

This book is highly and unreservedly recommended to all who wish to learn the fundamentals of human genetics.

Ferdinand Bertram
1894—1960

Dr. med. Hellmut Otto, Munster, Germany

Dr. Ferdinand Bertram died in Hamburg on Oct. 28, 1960, at the age of sixty-six. He was one of the most prominent German specialists in diabetes and played a leading role in the development of the modern therapy of diabetes.

Dr. Bertram was born in Hamburg on Jan. 3, 1894. After completing his studies in Natural Sciences and Medicine at the Universities of Marburg and Hamburg, he worked for a few years as Assistant in the Institute of the famous Hamburg physiologist and pharmacologist Bornstein. In his first publication in 1923 he dealt with problems of blood sugar regulation. In 1925 Dr. Bertram began to work as a clinician. In 1932 he became a lecturer and then Assistant Professor in the Medical Faculty of the University of Hamburg. At the same time he became the director of the II. Medical Clinic of the Hamburg-Barmbek Hospital, which became a widely known model diabetes clinic within a few years under his vigorous directorship.

Dr. Bertram's scientific work was centered on the therapeutic problems of diabetes mellitus. In 1925 he reported his investigations with insulin-protein mixtures in the *Klinische Wochenschrift.* He found that the effect of insulin could be enhanced and prolonged by the addition of protein, and in this way described the principle of action of "depot" insulin for the first time a decade before Hagedorn.

In 1927 he was one of the first in Germany to object to the very high fat and low carbohydrate diabetic diets which were generally accepted at that time. This unphysiological form of nutrition was developed in the preinsulin era, and was continued even with insulin therapy in spite of the fact that it was fundamentally out of date. It is hardly comprehensible today that Dr. Bertram with his postulate that diabetics should be given more carbohydrate and less fat, was strongly opposed for many years. Ignoring this opposition, in the following years Dr. Bertram developed a diet with less fat and richer in carbohydrates. He did not have to wait long for success: There were no more cases of ketonemia. His "modern diabetes therapy" succeeded gradually. It was proved to be right in the years 1943 to 1948 when the German people were nourished with a minimum of fat and protein but just enough carbohydrate. Diabetes in Germany had never before appeared so easy and uncomplicated as in those years. Today, a diet relatively rich in carbohydrate and poor in fat which is the optimal nutrition for diabetics, is generally accepted in Germany, and is a conditio sine qua non for the prevention of ketonemia, arteriosclerosis and the specific diabetic vascular complications.

From 1927 to 1930 Dr. Bertram worked extensively on the mode of action and the clinical application of Synthalin and other blood-sugar reducing guanidine derivatives. He was awarded the Dr. Martini prize of Hamburg University for his investigations. As is well known, the trial of oral diabetes treatment was soon given up at that time, partly as a result of his objections.

Almost thirty years later the successful clinical trial of the new oral "antidiabetic" drugs became the highlight of his career. In 1955—after the early death of Dr. Franke of Berlin, the inaugurator of the oral therapy of diabetes—Dr. Bertram worked out the clinical foundations of treatment with the sulfonylurea drugs. On Oct. 4, 1955, in a lecture given to the German Society for Digestive and Metabolic Diseases, and at the same time in a publication in the *Deutsche Medizinische Wochenschrift,* he reported the treatment of diabetes with sulfonylurea derivatives for the first time. In this first paper, all the essential points were mentioned concerning the clinical application of these drugs and the indications and the expectations of success in the different types of diabetes. In the following years Dr. Bertram's findings were confirmed everywhere and the details were completed.

Dr. Bertram's book *Die Zuckerkrankheit* (*Diabetes*) has been the standard work on diabetes in the German language since 1934. He also wrote the chapter on clinical diabetes in the new edition of Thannhauser's *Textbook of Metabolism and Metabolic Diseases* which is now also available in English. The *ABC für Zuckerkranke* (*ABC for Diabetics*), which he wrote twenty years ago for his patients, is the most widely distributed book of information for diabetics in German. The tenth edition of this book, which is the last

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but one, he dedicated to Dr. Joslin, his respected friend for many years, for his ninetieth birthday.

In May 1960 Dr. Bertram directed the International Biguanide Symposium at Aachen, Germany, with great vitality. A few days later he was obliged to go to bed with a severe illness.

In spite of this he still found the strength to make a complete revision of his textbook on diabetes for the new, fifth edition. He died on October 28 after a full life, and friends, colleagues and diabetic patients gave him the last words of appreciation and gratitude at his funeral.

ABSTRACTS

Appelman, David H. (Diabetes Clinic, Jewish Chronic Disease Hosp.; & Endocrinology Clinic & Dept. of Med., Beth-El Hosp., Brooklyn, N. Y.): KIMMELSTIEL-WILSON SYNDROME. New York J. Med. *61*:1518-22, May 1, 1961.

The author reviews present-day knowledge regarding the Kimmelstiel-Wilson syndrome, with special attention to and discussion of pathology, experimental production of lesions of diabetic glomerulosclerosis, the specific vascular lesion of diabetes, and the complication of nephrotic syndrome which may develop. Prognosis and treatment are also reviewed. C.A.R.

Arney, Glen K.; Pearson, Elinor; and Sutherland, Anne B. (U. S. Army Surg. Res. Unit, Brooke Army Med. Center, Fort Sam Houston, Tex.): BURN STRESS PSEUDODIABETES. Ann. Surg. *152*:77-90, July 1960.

Two patients exhibited hyperglycemia, glycosuria and marked dehydration following severe third-degree burns. In one case, impaired glucose tolerance returned to normal after recovery. Dehydration was primarily due to glycosuria and diuresis, and was accompanied by elevation of the nonprotein nitrogen, hemoglobin and hematocrit, serum sodium and chloride. Hyperglycemia was related to adrenal cortical hormone release, in the presence of forced feeding and dehydration. Five of eight other patients having hyperglycemia following burns were noted to have a family history of diabetes. The mechanism of the pseudodiabetes involved diminished peripheral insulin effect or impaired insulin secretion. A.R.C., Jr.

Axelsson, J.; Bueding, E.; and Bülbring, Edith (Dept. of Pharmacol., Univ. of Oxford, Oxford, England): THE INHIBITORY ACTION OF ADRENALINE ON INTESTINAL SMOOTH MUSCLE IN RELATION TO ITS ACTION ON PHOSPHORYLASE ACTIVITY. J. Physiol. *156*:357-74, April 1961.

The action of adrenalin on smooth muscle was studied in tenia coli of guinea pigs. In Ringer's solution containing glucose, adrenalin caused hyperpolarization of the cell membrane, stopped spontaneous spike activity, and abolished the conducted response to electrical stimulation. Adrenalin increased the phosphorylase activity of the smooth muscle. As the glycogen content of the tissue became depleted during incubation in glucose-free medium, the effect of adrenalin on the cell membrane became gradually less; after marked depletion of glycogen, the effect of adrenalin was reversed — it caused depolarization and initiated spikes. It is suggested that adrenalin may have opposite effects upon the cell membrane depending upon whether or not sufficient glycogen is present to act as a source of additional energy. H.T.N.

Baker, R. David; Searle, Gordon W.; and Nunn, Arthur S. (Dept. of Physiol., Coll. of Med., State Univ. of Iowa, Iowa City, Ia.): GLUCOSE AND SORBOSE ABSORPTION AT VARIOUS LEVELS OF RAT SMALL INTESTINE. Am. J. Physiol. *200*: 301-04, February 1961.

The passage of glucose and sorbose across the wall at various levels of rat small intestine has been studied in vitro. "Uphill" glucose transport was demonstrated in the jejunum and upper half of the ileum but could not be seen in the terminal quarter of small intestine. Aerobically, in the mid-jejunum glucose absorption from the mucosal solution was about 5.5 times as rapid as in the terminal ileum; the aboral decline in absorptive activity, however, occurred only in the lower half of the small intestine; the upper half absorbed glucose at a nearly constant rate throughout. No aboral decline for rate of glucose transfer into the serosal solution was noticed in the upper three-quarters of small intestine, but in the lower quarter the drop was precipitous.

The effects of hypoxia and fluoride poisoning on glucose movements were much more severe in the upper than in the lower portions of small intestine. Fluoride (48 mM) completely abolished the absorptive activity gradient for glucose along the small intestine. Movements of sorbose, which is passively absorbed, were quite similar in rate to the fluoride-inhibited glucose movements.

These observations indicate that the intestinal absorption gradient for glucose probably results from the relative distribution along the intestine of the same rate-limiting component of the active transport system for glucose. L.S.S.

Bassett, E. W.; Beiser, S. M.; and Tanenbaum, S. W. (Dept. of Microbiology, Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.): PURIFICATION OF ANTIBODY TO GALACTOSYL-PROTEIN CONJUGATES. Science *133*:1475-76, May 12, 1961.

A rapid procedure for purification of rabbit antibody to B-D-galactosylphenylazo-bovine serum albumin is described. Antibody was precipitated with a heterologous antigen and was then dissociated from the precipitated complex with hapten. The antigen was partially removed by *p*H adjustment, and antibody was separated from residual antigen and from hapten by partition chromatography on Sephadex G-25. The antibody, obtained in 50 to 55 per cent over-all yield, was 96 per cent reactive with homologous antigen. It appeared to consist of 95 per cent of a single component, based upon physicochemical measurements. L.S.S.

Beatty, Clarissa H.; Marcó, Alicia; Peterson, Ruth D.; Bocek, Rose Mary; and West, Edward S. (Dept. of Biochemistry, Univ. of Oregon Med. Sch., Portland, Oreg.): ACETOACETIC ACID METABOLISM BY SKELETAL MUSCLE FIBERS FROM CONTROL AND DIABETIC RATS. J. Biol. Chem. *235*:2774-77, October 1960.

Acetoacetic acid uptake by diaphragm and adductor muscle fibers was decreased in pancreatectomized diabetic rats as compared to control rats. Uptake could be enhanced by adding glucose and insulin in both groups. Conversion of acetoacetate-3-C-14 to C-14O_2 was decreased in the diabetic animals. There was an increase in manufacture of lactate after addition of acetoacetate to the medium. A.R.C., Jr.

Boley, Scott J.; Lin, Jane; and Schiffmann, Albert (Pediat. Surg. Div. and Dept. of Pediatrics, Jewish Hosp. of Brooklyn, Brooklyn, N. Y.): FUNCTIONING PANCREATIC ADENOMAS IN INFANTS AND CHILDREN. Surgery *48*:592-605, September 1960.

Seventeen cases of pancreatic adenoma in children less than fifteen years of age were collected from the literature, and one case was added. Most common symptoms were convulsions, unconsciousness, drowsiness, behavior changes and urinary incontinence. One third of the patients were overweight, and four had neurological signs. Whipple's triad, which includes the presence of attacks following fasting or exertion, blood glucose less than 50 mg. per 100 ml., and relief of symptoms by sugar, was noted in thirteen of seventeen children. Fasting blood glucose ranged between 11 and 50 mg. per 100 ml., and seven of ten glucose tolerance curves were flat with frequent subnormal values. Insulin tolerance tests showed increased resistance to insulin in four cases and hypoglycemic unresponsiveness. ACTH therapy is recommended for children less than four years of age, and surgical exploration for older patients. A.R.C., Jr.

Cohn, Clarence (Dept. Biochem., Med. Res. Inst. Michael Reese Hosp., Chicago, Ill.): EATING HABITS AND "METABOLIC" DISEASES. Illinois M. J. *119*:125-26, March 1961.

The rate at which foodstuffs become available to cells influences their metabolic activities. The laboratory rat ordinarily consumes small amounts of food over the twenty-four hour period. Forced feeding changes him to a meal eater and results in deposition of more body fat and less protein than would be the case if he fed normally. Forced feeding increased urinary sugar and nonprotein nitrogen in diabetic rats. Meal eating in chickens (normally nibblers) resulted in increased levels of serum cholesterol and a sevenfold increase in the incidence of coronary atherosclerosis. Experimentally elevated levels of serum cholesterol fell more slowly and atherosclerotic lesions healed more slowly when animals were meal-fed during regression studies.

Rate of food ingestion may influence human metabolism and could play a significant role in "metabolic" diseases. H.L.W.

Corvilain, J.; and Tagnon, R. (Clinique Médicale, Hôpital Saint-Pierre, Université Libre de Bruxelles, Brussels, Belgium): EFFECTS OF FRUCTOSE INFUSION ON GLUCOSE UPTAKE AND CIRCULATING INSULIN-LIKE ACTIVITY IN NORMAL MEN. J. Physiol. *155*:337-42, February 1961.

An intravenous infusion of fructose, 100 gm. in one hour, was given to normal subjects. The rate of glucose uptake from the blood stream was determined in nine subjects immediately before and after the infusion, by measuring the rate of disappearance of a small glucose load given by rapid intravenous injection. Blood glucose was determined by the method of Shaffer and Hartman, and a correction was applied for fructose determined by a separate chemical method. Blood fructose rose above 90 mg. per 100 ml., but blood glucose did not rise during the infusion. The calculated rate of glucose uptake from the blood stream was increased by fructose infusion. In four other subjects no increase in serum insulin-like activity was found after a fructose infusion, using an in vitro assay with adipose tissue. H.T.N.

Csáky, T. Z.; Hartzog, H. G., III; and Fernald, G. W. (Dept. of Pharmacol., The Univ. of North Carolina Sch. of Med., Chapel Hill, N. C.): EFFECTS OF DIGITALIS ON ACTIVE INTESTINAL SUGAR TRANSPORT. Am. J. of Physiol. *200*:459-60, March 1961.

Ouabain (10^{-6} moles) and thevetin (10^{-7} moles) completely inhibit the active transport of 3-methylglucose across the intestine of the frog in vitro. Acetylstrophanthidin has a weak inhibitory action. These results suggest a possible link between active ionic and sugar transport mechanisms, since digitalis inhibits the active transport of sodium across cells in the red cell, kidney and frog skin. L.S.S.

Davis, Joseph R. (Dept. of Pharmacol., Univ. of Illinois, Coll. of Med., Chicago, Ill.): ORAL ANTIDIABETIC AGENTS: A REVIEW OF THEIR PHARMACOLOGY, MECHANISM OF ACTION, AND CLINICAL USE. Illinois M. J. *119*:127-30, March 1961.

The history, mechanism of action, and clinical use of both the sulfonylureas, and guanidine derivatives is discussed. Potency, excretion, and dosage of the three currently available drugs — tolbutamide, chlorpropamide, and phenformin — are tabulated. H.L.W.

DiPietro, David L.; and Weinhouse, Sidney (Inst. for Cancer Res. and Chemistry Dept. of Temple University, Philadelphia, Pa.): HEPATIC GLUCOKINASE IN THE FED, FASTED, AND ALLOXAN-DIABETIC RAT. J. Biol. Chem. *235*:2542-50, September 1960.

Glucose 6-phosphatase was removed from homogenized rat liver by means of ultracentrifugation at 100,000 x g. This permitted assay of hepatic glucokinase by coupling with glucose 6-phosphate dehydrogenase and TPN reduction. Fasted or alloxan diabetic rats exhibited a marked reduction in hepatic glucokinase activity. A.R.C., Jr.

Ditschuneit, H.; Pfeiffer, E. F.; and Rossenbeck, H. G. (I. Medizinischen Universitäts Klinik Frankfurt a.M., Frankfurt, Germany): THE EFFECT OF SULFONYLUREA-COMPOUNDS AND OF BIGUANID (DBI) ON THE CARBOHYDRATE METABOLISM OF THE ISOLATED FAT PAD AND DIAPHRAGM OF THE RAT. III. CONCERNING THE DETERMINATION OF INSULIN IN THE BLOOD BY MEANS OF THE EPIDIDYMAL FAT PAD IN THE RAT WITH THE HELP OF TAGGED GLUCOSE. Klin. Wchnschr. *39*:71-76, Jan. 15, 1961.

The in vitro effect of several hypoglycemic sulfonylurea compounds (tolbutamide, metahexamide and chlorpropamide) and of phenformin (DBI) on the isolated epididymal fat pad of the rat was estimated both in buffer solution and in serum. The data were compared with those obtained with insulin.

The oxidation by the fat tissue of 1-C-14 glucose to C-14O_2 increased about seven times when 1,000 μU/ml. Crystalline Insulin were added. The same dose of insulin caused about a fourfold entrance of glucose into the fat tissue. In the diaphragm, insulin effect was considerably smaller and amounted to about 60 per cent. The sulfonylurea compounds had no sig-

nificant effect on the carbohydrate metabolism on either the epididymal fat or the isolated diaphragm; there was no significant increase of the rate of glucose entrance or of glucose oxidation, nor did the sulfonylurea compounds alter the insulin effect on these tissues. DBI, in a concentration of 20 gm./ml. in buffer solution, caused a marked inhibition of the glucose oxidation via the pentose-phosphate cycle and diminished the effect of insulin by about 85 per cent. In serum, DBI inhibited completely the insulin effect. There was, however, a small increase of glucose entrance in the tissues under the influence of DBI. These data confirm the marked difference in the mode of action of DBI and the sulfonylurea compounds; they support the concept that the sulfonylurea compounds have no peripheral effect and that they act via stimulation of insulin. (German) M.G.G.

Ehrlich, Joseph C.; and Ratner, Irving M. (Lebanon Hosp., New York, N. Y.): AMYLOIDOSIS OF THE ISLETS OF LANGERHANS: A RESTUDY OF ISLET HYALIN IN DIABETIC AND NONDIABETIC INDIVIDUALS. Am. J. Path. *38*:49-59, January 1961.

The nature of the hyaline material in the islets of Langerhans was investigated with the use of various staining technics; 49.5 per cent of ninety-one necropsies on diabetic patients over fifty years of age showed hyalinized islets. Only 3.9 per cent of 178 necropsies on nondiabetic individuals showed similar changes. The hyaline material showed the same characteristics as localized amyloid with respect to: (1) metachromasia with crystal violet, (2) binding of Congo red with dichromic birefringence, (3) periodic acid Schiff, (4) toluidine blue, and (5) Alcian blue.

Hyaline islets seem to be a form of localized amyloidosis occurring in the diabetes of maturity and said to be rare in juvenile diabetes. H.L.W.

Ellenberg, Max (Dept. of Med., The Mt. Sinai Hosp., New York, N. Y.): DIABETES MELLITUS: DIABETIC NEUROPATHY. New York State J. Med. *61*:1562-69, May 1, 1961.

The author reviews the subject of diabetic neuropathy with special emphasis on symptomatology, pathology, etiology, diagnosis and therapy. C.A.R.

Esmann, V. (Second Univ. Clinic of Internal Med., Kommunehospitalet, Aarhus, Denmark): THE GLYCOGEN CONTENT OF LEUKOCYTES FROM DIABETIC AND NONDIABETIC SUBJECTS. Scandinav. J. Clin. & Lab. Invest. *13*:134-39, 1961.

Blood from diabetic and nondiabetic subjects was collected in dextran with chilling, and the leukocytes were collected by centrifugation after the erythrocytes had settled. The subjects were in a fasting condition. Eleven of the diabetics had never received insulin, and insulin was withheld for one to four days from the others. The glycogen content of leukocytes from twenty-five nondiabetic resting subjects averaged 148 mg. (standard error 9 mg.) per 10^{10} myeloid leukocytes and in fourteen uncontrolled diabetic patients was significantly lower: 111 mg. (S.E. 10 mg.) per 10^{10} myeloid leukocytes. The glycogen level of leukocytes returned to a normal level after insulin therapy. The actual fraction of total leukocyte glycogen which was in the myeloid cells was not determined. H.T.N.

Ferguson, Harold A.; Coniglio, John G.; and Blood, Frank R. (Dept. of Biochemistry, Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): EFFECT OF DIETARY FAT ON METABOLISM OF CHOLESTEROL-26-C-14 IN THE RAT. Am. J. Physiol. *200*: 539-40, March 1961.

Rats were maintained for varying periods of time on a purified diet containing 20 per cent fat as hydrogenated cottonseed oil, corn oil or coconut oil and then injected intravenously with a tracer dose of cholesterol-26-C-14 in solution. No significant influence was observed by the type of fat ingested on the amount of C-14 converted to $C\text{-}14O_2$, excreted in feces, or retained in the nonsaponifiable fraction of several selected tissues. From 9 to 17 per cent of the administered dose of C-14 was expired as $C\text{-}14O_2$, and from 3 to 8 per cent was excreted in feces in seventy-two hours. The greatest amount of C-14 retained in the body was found in the liver (11-22 per cent), and in this organ the largest values were observed in those animals which had been on the diet the shortest length of time. L.S.S.

Fine, Melwyn B.; and Williams, Robert H. (Dept. of Med., Univ. of Washington Sch. of Med., Seattle, Wash.): EFFECT OF FASTING, EPINEPHRINE, GLUCOSE AND INSULIN ON HEPATIC UPTAKE OF NONESTERIFIED FATTY ACIDS. Am. J. Physiol. *199*:403-06, September 1960.

Hepatic uptake of nonesterified fatty acids (NEFA) was determined from the portal-hepatic NEFA difference and the hepatic blood flow. Hepatic NEFA uptake was found to increase with fasting and after administration of epinephrine. It was found to decrease after the administration of glucose and insulin. It was concluded that these factors did not directly influence the liver to alter its NEFA uptake but that the liver responded primarily to the concentration of NEFA in the blood entering it. W.R.K.

Fischer, Alfred E.; and Moloshok, Ralph E. (Dept. of Pediatrics, Mt. Sinai Hosp., New York, N. Y.): DIABETIC AND PREDIABETIC PREGNANCIES WITH SPECIAL REFERENCE TO THE NEWBORN: A FIVE-YEAR STUDY. J. Pediat. *57*:704-14, November 1960.

An analysis is presented of experience with infants born to ninety-seven diabetic women and to sixty-two women with histories suggesting the prediabetic state, corroborated by decreased tolerance to the ingestion of glucose. Methods of management of the mother and of the infant during the neonatal period are described. Careful long-term medical and obstetric attention exert a favorable influence upon fetal survival. Factors detrimental to the fetus of a diabetic mother are active in the five-year period immediately before the onset of maternal diabetes but do not appear to operate earlier. The outcome of pregnancies did not correlate with the duration of the diabetic state. In sixteen pregnancies among mothers with diabetes of twenty years' or more duration, thirteen of fourteen viable births survived. Other evidence indicates that the occurrence of fetal or perinatal death does not rule out the probability of a surviving infant from a subsequent pregnancy. W.R.K.

Fitzgerald, M. G.; Malins, J. M.; O'Sullivan, D. J.; and Wall, Mary (Diabetic Clinic Gen. Hos., Birmingham and Dept. of Med. Statistics, University of Birmingham, England): THE EFFECT OF SEX AND PARITY ON THE INCIDENCE OF DIABETES MELLITUS. Quart. J. Med. *30*:57-70, January 1961.

The relative age and sex specific incidence of diabetes mellitus in Birmingham is shown for 5,441 patients who first attended the diabetic clinic between 1949 and 1958.

The onset of diabetes is commoner in men than women before the age of forty. After forty as the incidence in both sexes increases, more women than men are affected. This differ-

ence is confined to married women and diabetes remains commoner in men than in single women at all ages.

The number of live births in 2,318 married diabetic women of known ages over forty-five is compared with those recorded in 7,608 married women of the same ages admitted to all departments of a general hospital. At all ages a greater percentage of the diabetic women have had four or more, especially seven or more, children.

The incidence of diabetes increases with each increase in parity; compared with that in nulliparae, it is about twice as common in women who have had three children, and six times as common in those who have had six or more. It is equally common in single and married nulliparous women. After the age of forty-five, and allowing for the excess of elderly women in the population, the excess of women having the disease is accounted for by those who have borne three or more children. J.A.

Fletcher, Lucian, Jr.; Torosdag, Seta; and Bryant, J. Marion (Dept. of Med., New York Univ. Post-Grad. Med. Sch. & Hypertensive Study Group, 4th (N.Y.U.) Med. Div., Bellevue Hosp., New York, N. Y.): STUDIES OF THE EFFECTS OF PHENMETRAZINE (PRELUDIN) IN CARDIOVASCULAR DISEASE. Am. J. Sc. *241*:491-502, April 1961.

Intravenous phenmetrazine (10 to 25 mg.) produced mild transient (five to ten minutes) elevations of blood pressure without the appearance of cardioacceleratory or irritating effects. Long-term oral administration (one to twenty-one months) of phenmetrazine, 25 mg. three times daily, to a group of hypertensive patients, did not produce significant changes in the blood pressure of the group as a whole or other undesirable cardiovascular effects while providing decrease in appetite and resultant weight loss.

It is suggested that the benefits of reduction in weight effected by an anorexic agent such as phenmetrazine in obese patients with cardiovascular disease, outweigh any conventional objections to its use. In practice, obese cardiac and hypertensive patients show no adverse effects from phenmetrazine in therapeutic dosage. L.S.S.

Flinn, Robert B.; Leboeuf, Bernard; and Cahill, George F., Jr. (Depts. of Med., Harvard Med. Sch. & Peter Bent Brigham Hosp.; & Baker Clin. Res. Lab., New England Deaconess Hosp., Boston, Mass.): METABOLISM OF C-14-LABELED SUBSTRATES IN KIDNEY CORTICAL SLICES FROM NORMAL AND ALLOXAN-DIABETIC RATS. Am. J. Physiol. *200*:508-10, March 1961.

The metabolism of C-14-labeled glucose, fructose, mannose and pyruvate was studied in kidney slices from normal and alloxan-diabetic rats. Labeled carbon was recovered in CO_2, glycogen and fatty acids. The rate of mannose metabolism was consistently slightly less than that of glucose, and on the other hand, the rate of fructose metabolism was consistently greater. In slices from alloxan-diabetic animals the metabolism of glucose and mannose was markedly reduced; that of fructose was only slightly decreased. No effect of insulin added in vitro could be demonstrated on glucose metabolism. Pyruvate oxidation to CO_2 was slightly increased in slices from diabetic animals. Furthermore, glucose production was increased sixfold by diabetes and, as calculated, 75 per cent of this glucose production could be accounted for by glucose synthesized from labeled pyruvate. The similarity between carbohydrate metabolism in liver and kidney was pointed out. L.S.S.

Goldner, Martin G.; and Weisenfeld, Shirley (Dept. of Med. of the Jewish Chronic Disease Hosp. and State Univ. of New York Downstate Med. Center, Brooklyn, N. Y.): PRESENT STATUS OF THE ORAL HYPOGLYCEMIC AGENTS. New York J. Med. *61*:1328-36, April 15, 1961.

The authors review the present status of oral hypoglycemic agents, including their chemical structure and pharmacology and mode of action. Clinical aspects and dosage as well as indications for substitution in place of insulin or combined treatment with insulin, as well as toxicity and side effects are also reviewed. C.A.R.

Graef, Irving (Dept. of Med., Lenox Hill Hosp., New York, N. Y.): ON CLASSIFYING DIABETES MELLITUS. New York J. Med. *61*:1734-38, May 15, 1961.

The author presents a clinical classification of diabetes mellitus, with relation to various etiologies. He also discusses the clinical syndromes of prediabetes, malignant vascular diabetes, juvenile and postmaturity diabetes, secondary diabetes and stress diabetes. He presents this classification as a working framework, subject to possible future change depending on its continued validity with the test of time and newer knowledge on the subject. C.A.R.

Hagen, Jean Himms (Dept. of Physiol., University of Manitoba Med. Sch., Winnipeg, Manitoba, Canada): EFFECT OF INSULIN ON THE METABOLISM OF ADIPOSE TISSUE FROM HYPERTHYROID RATS. J. Biol. Chem. *235*:2600-03, September 1960.

Rats made hyperthyroid by injection of thyroxine for twenty to forty days were killed and their epididymal fat pads removed. This adipose tissue when incubated with insulin took up glucose and produced CO_2 at a rate twice that of normal adipose tissue. Release of fatty acids was increased in hyperthyroid rats and insulin inhibited the release more than it did in normal rats. Thyroxine improved the ability of fat tissue to respond to insulin, and may also have increased fat synthesis under the influence of insulin. A.R.C., JR.

Hatch, Fred E., Jr.; and Parrish, Alvin E. (The Dept. of Medicine, District of Columbia Gen. Hosp. Div., George Washington Univ., Washington, D. C.): APPARENT REMISSION OF A SEVERE DIABETIC ON DEVELOPING THE KIMMELSTIEL-WILSON SYNDROME. Ann. Int. Med. *54*:544-49, March 1961.

The authors describe the clinical course of a patient with known diabetes of fourteen-years' duration and Kimmelstiel-Wilson's disease for two years before death. The patient had required up to 100 units of insulin at one time but had a distinct remission of the disease with the onset of clinical nephrosis. The patient at this point no longer required insulin and an oral insulin tolerance test was normal. Renal biopsy confirmed the diagnosis of diabetic nephropathy. S.B.B.

Izzo, Joseph L.; and Glasser, Stanley R. (Depts. of Med. & Radiation Biology, Univ. of Rochester Med. Center, Rochester, N. Y.): COMPARATIVE EFFECTS OF GLUCAGON, HYDROCORTISONE AND EPINEPHRINE ON THE PROTEIN METABOLISM OF THE FASTING RAT. Endocrinology *68*:189-98, February 1961.

The effect of glucagon on nitrogen catabolism was studied and compared with the effects of hydrocortisone and epinephrine. Both glucagon and hydrocortisone caused weight loss and increased urinary nitrogen, phosphorus, and creatinine.

Glucagon treated rats lost less body weight, tissue protein, and urinary phosphorus with a lower blood amino nitrogen than the hydrocortisone treated animals. The rate of nitrogen and

phosphorus loss slowed over five days in the former group and accelerated in the latter.

Glucagon and hydrocortisone produce protein catabolism in a different way. Glucagon directly promotes protein catabolism by the liver while hydrocortisone mobilizes amino acids from extrahepatic tissues.

Epinephrine depressed liver glycogen, as does glucagon, but did not cause deviation from control values for the other parameters of protein metabolism. The protein catabolic effect of glucagon is not a direct consequence of diminished hepatic glycogen. H.L.W.

Josimovich, J. B.; and Knobil, E. (Dept. of Physiol., Harvard Med. Sch., Boston, Mass.): PLACENTAL TRANSFER OF I^{131}-INSULIN IN THE RHESUS MONKEY. Am. J. Physiol 200:471-76, March 1961.

Pregnant rhesus monkeys were anesthetized, their uteri were exposed, and the interplacental vessels (fetal circulation) were cannulated without incision of the amnion. This procedure permitted simultaneous sampling of maternal blood, umbilical venous blood and umbilical arterial blood. I^{131}-labeled insulin injected into the maternal circulation was detected in umbilical venous plasma, within five minutes after the injection, by the use of a chromatographic procedure which permits the separation of I^{131}-insulin from other iodinated compounds. The concentration of I^{131}-insulin in fetal plasma did not exceed 20 per cent of that found concurrently in maternal plasma. A marked umbilical arterial-venous difference in I^{131}-insulin concentration was consistently observed. The concentration of iodinated degradation products of I^{131}-insulin was considerably higher in umbilical arterial plasma than in umbilical venous plasma, suggesting rapid degradation of the labeled hormone by the fetus. Conversely, the injection of I^{131}-insulin into the fetal circulation was followed by the appearance of significant quantities of the labeled hormone in the maternal circulation. These experiments lead to the conclusion that insulin can cross the primate placenta. L.S.S.

Kerkhof, Arthur C. (Minneapolis, Minn.): UNCONSCIOUSNESS WITH DIABETES. Journal-Lancet *81*:207-09, May 1961.

Causes and treatment of insulin-induced hypoglycemia and diabetic ketoacidosis are discussed as two instances that caused unconsciousness in the diabetic. H.L.W.

Kinsey, V. E.; and Reddy, D. V. N. (Kresge Eye Inst., Detroit, Mich.): TRANSPORT OF GLUCOSE ACROSS BLOOD-AQUEOUS BARRIERS AS AFFECTED BY INSULIN. J. Physiol. *156*:8-16, April 1961.

Glucose can pass from the blood stream into the aqueous humor of the posterior chamber of the eye; glucose in the anterior chamber can arise from the posterior chamber, or from the blood stream directly. The rates of these processes were studied in normal or alloxan-diabetic rabbits, with or without the administration of exogenous insulin. In some experiments, a tracer dose of glucose-U-C-14 was given to rabbits intravenously, and at intervals samples of blood, and fluid from the anterior and posterior chambers of the eye, were collected; the total nonvolatile C-14 content was taken as a measure of glucose-C-14 concentration. The rate of exchange of glucose-C-14 for nonlabeled glucose in the eye was thus measured, and taken as an index of the rate of accumulation of glucose. In other experiments, blood glucose was raised by the parenteral injection of nonlabeled glucose, and the net rate of accumulation of glucose in the chambers of the eye was determined with glucose oxidase. No significant difference was found with either method of measurement in the eye chambers of any of the animal groups (diabetic or insulin-treated). H.T.N.

Kipnis, David M.; and Cori, Carl F. (Depts. of Biological Chemistry and Medicine, Washington Univ. Sch. of Medicine, St. Louis, Mo.): STUDIES OF TISSUE PERMEABILITY. VI. THE PENETRATION AND PHOSPHORYLATION OF 2-DEOXYGLUCOSE IN THE DIAPHRAGM OF DIABETIC RATS. J. Biol. Chem. *235*: 3070-75, November 1960.

The glucose analogue, 2-deoxyglucose, was phosphorylated at an impaired rate by intact rat diaphragm prepared from diabetic rats The effect was related directly to the severity of the diabetes. In the absence of insulin, penetration of this sugar was rate-limiting, and this could be corrected by the addition of insulin. Phosphorylation was corrected by adrenalectomy but not by insulin. D-xylose penetration was normal. Accumulation of free sugar is due to decreased phosphorylation, under control of the pituitary-adrenal system. Penetration of sugar into the muscle cell and regulation of the blood glucose are under insulin control. A.R.C., JR.

Knobil, E.; Best, G. R.; and Goodman, H. M. (Dept. of Physiol., Harvard Med. Sch., Boston, Mass.): THE HYPOGLYCEMIC ACTION OF SIMIAN AND BOVINE GROWTH HORMONE IN HYPOPHYSECTOMIZED RHESUS MONKEYS. Endocrinology *68*:723-25, April 1961.

Fasting hypophysectomized rhesus monkeys developed hypoglycemia after receiving simian growth hormone. The fall in blood sugar was not seen in fed-hypophysectomized or fasted normal monkeys. Bovine growth hormone was ineffective in the monkey.

The insulin-like action of growth hormone is an inherent property of the molecule itself since it is "species specific." H.L.W.

Kritchevsky, David; Staple, Ezra; Rabinowitz, Joseph L.; and Whitehouse, Michael W. (Wistar Inst. of Anat. & Biology; Dept. of Biochem., Univ. of Pennsylvania Sch. of Med.; & Radioisotope Serv.; VA Hosp., Philadelphia, Pa.): DIFFERENCES IN CHOLESTEROL OXIDATION AND BIO-SYNTHESIS IN LIVER OF MALE AND FEMALE RATS. Am. J. Physiol. 200: 519-22, March 1961.

Female rat liver mitochondria oxidized cholesterol-26-C-14 and sodium pyruvate-2-C-14 to $C\text{-}14O_2$ to a much greater extent (per mg. N) than did male rat liver mitochondria. Mitochondrial preparations from livers of castrated, estrogenized or castrated-estrogenized male rats all oxidized cholesterol-26-C-14 to a greater extent than did liver preparations from normal male rats. No differences were observed in the oxidation of sodium octanoate-1-C-14. The serum and liver cholesterol levels of the feminized rats were higher than those of the intact males. Biosynthesis of cholesterol from sodium acetate-2-C-14 by male rat liver homogenates was significantly lower than biosynthesis by liver homogenates from normal female rats or gonadectomized rats of both sexes. The rate of biosynthesis from mevalonic acid-2-C-14 by liver homogenates from castrated male rats was much higher than in homogenates of oophorectomized females or intact males or females. Differences in sex or gonadectomy had no effect on biosynthesis of fatty acids from sodium acetate-2-C-14. L.S.S.

Lakin, Mervyn; Bradley, Robert F.; and Bell, George O. (Woman's Hosp., Detroit, Mich.; & Lahey Clinic, Boston, Mass.): ACUTE HYPERTHYROIDISM IN SEVERE DIABETIC KETOACIDOSIS. Am. J. M. Sc. *241*:443-447, April 1961.

The authors report five cases of coexisting severe diabetic ketoacidosis and hyperthyroidism in over 50,000 diabetic patients seen at the Joslin Clinic Service during the years 1923-1960, making a total of at least nine reported occurrences of this combination in the medical literature. Four of the five were women, and the one death (in 1924) resulted from sepsis twenty-two days after admission.

A high index of suspicion facilitates making the diagnosis of hyperthyroidism in the presence of diabetic ketoacidosis. Evaluation of the cause for ketoacidosis or coma, suggestive prior symptoms of hyperthyroidism, fever during coma in the absence of infection, persistent tachycardia not responding to correction of coma or to digitalis therapy, and thyromegaly should aid in its early detection.

Ketoacidosis responded promptly to adequate treatment with insulin and intravenous fluids. Although antithyroid treatment with Lugol's solution was used in only two of the reported cases during the phase of recovery from diabetic coma, the prompt recognition and energetic treatment of acute hyperthyroidism may assist in prompt response of the ketoacidosis and will certainly facilitate subsequent diabetic management.

In the most recent case it is possible that Graves' disease was in part precipitated by thyroid medication during a recent pregnancy. L.S.S.

Mabry, C. Charlton; DiGeorge, Angelo M.; and Auerbach, Victor H. (Depts. of Pediat. and Physiol. Chem., Temple Univ. Sch. of Med., and St. Christopher's Hosp. for Children, Philadelphia, Pa.): LEUCINE-INDUCED HYPOGLYCEMIA: I. CLINICAL OBSERVATIONS AND DIAGNOSTIC CONSIDERATIONS. J. Pediat. 57:526-38, October 1960.

Two infants with leucine-sensitive hypoglycemia are presented. A standard intravenous leucine tolerance test has been devised and utilized in the study of these two infants and of euglycemic and other hypoglycemic children who were not leucine sensitive. The leucine-sensitive children consistently had a decline in blood sugar levels of greater than 50 per cent twenty to forty minutes after administration of the leucine. The diagnosis of leucine-sensitive hypoglycemia and management of infants who have it are discussed. W.R.K.

Mabry, C. Charlton; DiGeorge, Angelo M.; and Auerbach, Victor H. (Depts. of Pediat. and Physiol. Chem., Temple Univ. Sch. of Med., and St. Christopher's Hosp. for Children, Philadelphia, Pa.): LEUCINE-INDUCED HYPOGLYCEMIA: II. STUDIES CONCERNING OTHER AMINO ACIDS AND LEUCINE METABOLITES. J. Pediat. 57:539-47, October 1960.

Two infants with leucine-sensitive hypoglycemia have been studied to elucidate the specificity and mechanism of action of L-leucine. Sixteen other naturally occurring L-amino acids have been administered intravenously and tyrosine and tryptophan by gavage. Of these amino acids, only L-leucine consistently induced hypoglycemia. D-leucine also produced hypoglycemia when given intravenously. Alpha-ketoisocaproic and isovaleric acids were administered intravenously; the former induced hypoglycemia whereas the latter did not. Monocarboxylic ketoacids in the urine were measured following L-leucine or alpha-ketoisocaproic acid loading. The recovery of ketoacids in the urine was of a low order, similar to that of a control subject, indicating no metabolic defect in the conversion of alpha-ketoisocaproic acid to isovaleric acid. Preliminary evidence is presented that leucine administration to affected patients results in increased circulating insulin levels. W.R.K.

Martz, B. L. (Marion County Genl. Hosp., Indianapolis, Ind.): DIET AND ATHEROSCLEROSIS. Illinois M. J. *119*:188, April 1961.

Dietary restriction of saturated fat is suggested as a means of diminishing serum cholesterol levels and atherosclerosis. H.L.W.

McElroy, William T., Jr.; and Spitzer, John J. (Dept. of Physiol., Hahnemann Med. Coll. & Hosp., Philadelphia, Pa.): EFFECTS OF ADRENERGIC BLOCKING AGENTS ON PLASMA FREE FATTY ACID CONCENTRATIONS. Am. J. Physiol. *200*:318-22, February 1961.

Based upon the concept that plasma free fatty acids (FFA) are lipids in transport from adipose tissue to other tissues and that epinephrine and norepinephrine increase both the rate of FFA release from adipose tissue and its concentration in plasma, the authors report studies on the effect upon these metabolites exerted by adrenergic or ganglionic blocking agents. To blockade tonic sympathetic influences on FFA plasma concentrations, or responses by adipose tissue to administered amines, dogs rereceived dibenzyline, dibenamine, ergotamine (Gynergen) or trimethaphan (Arfonad). Plasma FFA and glucose in femoral vessels and hepatic and portal veins were determined. Estimated hepatic plasma flow (EHPF) and mean blood pressures were recorded. Unanesthetized, untreated dogs often showed FFA responses to injected amines that were quantitatively different from previous testing. FFA responses in anesthetized animals were more reproducible. Dibenzyline elevated FFA and glucose levels and lowered blood pressure. Trimethaphan elevated FFA concentrations and decreased EHPF, glucose and blood pressure. Ergotamine decreased FFA, blood pressure and EHPF. Dibenzyline or dibenamine failed to block glucose or FFA responses to injected catecholamines, but ergotamine usually decreased hyperglycemic and occasionally FFA responses. Trimethaphan did not prevent FFA or glucose changes in dogs made hypercapnic. Like the hyperglycemic response, FFA release by adipose tissue is difficult to block even with large doses of blocking agents. L.S.S.

Metz, Robert; and Best, Charles H. (Banting and Best Dept. of Med. Res., Univ. of Toronto, Toronto, Ont., Canada): INSULIN AND GLUCAGON: A REVIEW. Practitioner *185*:593-601, November 1960.

Ever since 1921 successive attempts to delineate the place of insulin in the physiology of the mammalian organism have been characterized by marked shifts in emphasis as new aspects of its action have come to light. The pace of discovery has been accelerating in recent years, but much remains to be elucidated. By what mode, or modes, of action insulin brings about its bewildering variety of effects will not, in all probability, be known for many years. Neither the mechanism of its secretion from the beta cell, nor the factors concerned with "resistance" to the hormone are well understood. The discovery of glucagon, or at least of a hyperglycemic-glycogenolytic contaminant of insulin, was made as long ago as 1923, but only in the last twelve years has interest in this substance quickened. Nevertheless, in one respect information concerning glucagon has outstripped that on insulin, in that an action of glucagon on one specific enzymatic reaction has been clearly demonstrated. As with insulin, however, new metabolic effects of glucagon have

recently been discovered, and their mode of action and relative importance await elucidation. This review is designed to present a survey of the current situation with respect to both of these secretions of the islets of Langerhans. W.R.K.

Moore, Clemmie Everley; Kay, Jacob L.; Desmond, Murdina M.; and Dutton, Robert V. (Newborn Study Unit, Dept. of Pediatrics, Baylor Univ. Coll. of Med., and Nursery Services of Jefferson Davis, Hermann, and St. Luke's Hospitals, and Junior League Well Baby Clinic of Hermann Hosp., Houston, Tex.): TRANSITIONAL DISTRESS IN INFANTS OF DIABETIC MOTHERS. J. Pediat. 57:824-35, December 1960.

The clinical behavior of twenty-eight consecutive liveborn infants of diabetic mothers has been described. The patients were divided into three groups on the basis of symptomatology: Group A — No observed distress, 7 cases; Group B — Transient symptomatology without definite clinical pattern, 5 cases; Group C — Transitional distress (cardiopulmonary and neurological distress), 16 cases.

The symptomatology shown by the Group C infants occurred in an orderly and predictable sequence. The type of symptomatology, sequence, and complications were similar to those previously described in infants with prolonged umbilical cord pulsations and termed transitional distress. It was concluded that the cardiopulmonary and neurological symptoms of infants of diabetic mothers may also be termed transitional distress. Transitional distress in infants of diabetic mothers differed from transitional distress in infants with prolonged umbilical cord pulsations in two respects: The duration of distress was longer, and more of the infants were rated as "good" at birth. The severity of maternal diabetes appeared to be the most important factor related to the presence of distress in the infant. W.R.K.

Narahara, H. T.; Ozand, Pinar; and Cori, Carl F. (Dept. of Biological Chemistry, Washington University Sch. of Medicine, St. Louis, Mo.): STUDIES OF TISSUE PERMEABILITY. VII. THE EFFECT OF INSULIN ON GLUCOSE PENETRATION AND PHOSPHORYLATION IN FROG MUSCLE. J. Biol. Chem. 235:3370-78, December 1960.

Penetration of glucose into isolated frog sartorii was enhanced by insulin. The rate of intracellular glucose transfer depended on the time required for alteration of membrane permeability by insulin. Glucose penetration had a greater rate constant than did glucose efflux from the muscle. Kinetics of glucose transport across cell membranes are discussed. A.R.C., JR.

Nowell, John F.; and Ellis, George S. (Dept. of Ophthalmology, Louisiana State Univ. Sch. of Med., New Orleans, La.): LIGHT COAGULATION IN THE TREATMENT OF RETINAL DISEASE. South. M. J. 54:395-402, April 1961.

The authors describe the instrument developed since 1945 by Professor G. Meyer-Schwickerath of West Germany and found to be a valuable adjunct in the therapy of incipient retinal detachment. The instrument employs high intensity visible light to fuse the retina and choroid in minute circumscribed scars. Other uses are becoming manifest in the treatment of intraocular tumors, chorioretinitis, certain skin lesions, and in performing optical iridotomy. The principle is that of the burning glass used to focus the sun's rays to ignite wood. The source of light in this apparatus is an electric arc enclosed in a quartz tube containing xenon gas under high pressure. This light is passed through an optical system of lenses and emerges via an ophthalmoscope mirror. The operator can direct a filtered portion of the beam into the patient's eye, and when it is accurately placed on the fundus he can switch on the full beam for a fraction of a second. The normal refractive power of the eye focuses the light on the retina, and a minute chorioretinal burn is produced. Several applications will seal off the area under treatment. L.S.S.

Paullada, Juan Jose; and Luna del Villar, Jose (Dept. of Endocrinology & Metabolism of the Genl. Hosp. of Mexico City, Ward 5, Mexico City, D.F., Mexico): CLINICAL EVALUATION OF TOLCYCLAMIDE IN TREATMENT OF DIABETES MELLITUS. Metabolism 10:221-30, March 1961.

The authors report the use of a new drug, 1-cyclohexyl-3p-tolylsulfonylurea (tolcyclamide) in thirty-two diabetics ranging in age from twenty-one to seventy-two years. Fifteen of these patients had diabetes for less than five years and seventeen for more than five years. Each group contained one juvenile diabetic. In the first group, 0.5 gm. of this drug, tolcyclamide, t.i.d., produced normal blood sugar levels in eleven patients, and doubling the dose reduced the fasting blood sugar to essentially normal levels in all of the remaining patients except the juvenile diabetic. In the latter group, the fasting blood sugar became normal in only six patients on 1.5 gm. of the drug daily, and doubling this dose controlled only two additional patients. Similarly, the juvenile diabetic in this group did not respond satisfactorily. Blood counts, urinalysis, and PSP clearance were unchanged during treatment and no allergenic reactions were noted. This new drug appears to control a greater percentage of recent onset diabetics than do other sulfonylurea derivatives, and will probably be useful in the treatment of such patients. C.A.R.

Pinter, Gabriel G.; and Bailey, Robert E. (Depts. of Physiol. and Anat., Univ. of Tennessee, Memphis, Tenn.): ANEMIA OF RABBITS FED A CHOLESTEROL-CONTAINING DIET. Am. J. Physiol. 200:292-96, February 1961.

The anemia developed by rabbits fed a standard rabbit chow diet with added cholesterol and carrier fat seems to be of the hemolytic type. A striking increase in reticulocyte count, the appearance of nucleated red cells of differing maturity in the peripheral circulation, anisocytosis, anisochromia and erythrocytes with punctate basophilia are characteristic findings on stained films and are the first symptoms of the progressing anemia. The red cell count decreases and reaches the lowest level at about the eighth to twelfth week of exposure of the animals to cholesterol. Cr-51 survival studies revealed a shortened T 1/2 (3.7 days compared to twelve days), which is the established normal value under these conditions. The results of cross-transfusion studies are interpreted as the indication that the anemia is developed as the consequence of the production of red cells with decreased resistance. The data indicate that cholesterol alters the function of the erythropoetic tissue. L.S.S.

Radding, Robert S.; and Zimmerman, Stanley J. (Dept. of Med., Hermann Hosp., Houston, Tex.): PHENETHYLDIGUANIDE—COMPARATIVE STUDY OF TABLETS AND TIMED-DISINTEGRATION CAPSULES. Metabolism 10:238-45, March 1961.

The hypoglycemogenic efficacy of a single 100 mg. tablet and a 100 mg. time-disintegration capsule of phenethyldiguanide (phenformin) was compared in ten hospitalized female diabetics receiving approximately 2000-calorie diets. A single dose of DBI in a time-distintegration capsule was generally more effective than in tablet form, particularly when the fasting blood sugar level was below 200 mg. per 100 ml. DBI tablets acted for six to eight hours whereas the drug in capsule form exerted a hypoglycemic effect for fourteen

hours. Nausea and vomiting were less common with the capsule than with the tablet. In view of these findings, the authors feel that time-disintegration capsules may be the dosage form of choice. C.A.R.

Remein, Quentin R.; and Wilkerson, Hugh L. C. (Chronic Dis. Program, Div. of Special Health Services, Bureau of State Services, Public Health Service, United States Dept. of Health, Education, and Welfare, Washington, D. C.): THE EFFICIENCY OF SCREENING TESTS FOR DIABETES. J. Chron. Dis. *13*:6-21, January 1961.

A total of 595 ambulatory medical outpatient department patients were screened for diabetes by previous urine and blood tests. The latter were tested fasting and at various times after a test meal and 100 gm. of glucose orally. Detailed analysis was made of many factors for both sensitivity and specificity.

Certain tentative conclusions were reached: Somogyi and Folin-Wu blood sugar tests were preferred over the anthrone method. Specific glucose oxidase method of urine testing and a two-hour postprandial (with 74 gm. of carbohydrate in the meal) urine was recommended. Postprandial blood sugar tests were preferred at one to two hours. The blood-sugar methods screened out more than 1.5 times as many cases of diabetes than urine tests which also were performed under optimum conditions. S.B.B.

Robinson, B. H. B.; and Wright, P. H. (Dept. of Med., Postgraduate Med. Sch., London, W. 12; and Dept. of Chem. Path., Guy's Hosp. Med. Sch., London, S.E. 1, England): GUINEA-PIG ANTI-INSULIN SERUM. J. Physiol. *155*:302-10, February 1961.

Antibodies to beef insulin were produced in guinea pigs by injecting the antigen subcutaneously with Freund's adjuvant. The effect of several modifications of immunization procedure was tested. Antiserum caused hyperglycemia when injected intravenously into rats; this effect could be counteracted by administration of bovine, pig or sheep insulin. Antiserum also agglutinated sheep red cells that had been conjugated with insulin. H.T.N.

Savitsky, J. Philip (Lucy & Henry Moses Res. Labs., Med. Div., Montefiore Hosp., New York, N. Y.): PROLONGED ALTERATION OF SERUM CHOLESTEROL INDUCED BY DEOXYRIBONUCLEIC ACID IN RABBITS. Am. J. Physiol. *200*:541-44, March 1961.

Desoxyribonucleic acid (DNA) was carefully prepared in a highly purified, undenatured form from beef liver. Each rabbit of a group of twenty rabbits received a single injection of 1 mg. of this heterologous DNA. The mean serum cholesterol declined significantly within four months and was maintained at the new lower level with no return to control values for at least two years. This change in the mean reflected the decline in cholesterol levels of those animals with high normal values. The serum phospholipids responded similarly but no changes were observed in the serum neutral fat concentrations. Injection with varying quantities of DNA showed that the time required for changes to appear depended upon the amount of DNA injected. The mechanism for this reduction in serum cholesterol is not known but the possibility is suggested that the administered DNA alters the genetically controlled factors which determine the serum cholesterol level. In brief, DNA changes the "cholesterostat." L.S.S.

Schlesinger, F. G.; and van Gastel, C. (Med. Dept., University Hosp., Utrecht, The Netherlands): POSSIBLE AGGRAVATION OF ABDOMINAL SYMPTOMS BY TOLBUTAMIDE IN A PATIENT WITH DIABETES AND HEPATIC PORPHYRIA. Acta med. Scandinav. *169*:433-35, April 1961.

A diabetic patient suffered with acute attacks of hepatic porphyria with skin lesions, abdominal colic and massive excretion of uroporphyrins probably precipitated by tolbutamide. The symptoms disappeared and the uroporphyrin excretion decreased after the drug was discontinued. B.F.K.

Schwartz, James F. (New York Neurological Inst., Columbia-Presbyterian Med. Center, New York, N. Y.): TOLBUTAMIDE-INDUCED HYPOGLYCEMIA IN PARKINSON'S DISEASE: A CASE REPORT. J.A.M.A. *176*:106-09, April 15, 1961.

The use of tolbutamide in a seventy-three-year-old nondiabetic man with Parkinson's disease resulted in severe and prolonged hypoglycemia, precipitating coma and focal neuronal damage. The dangers inherent in therapy of elderly patients with hypoglycemic agents are discussed. B.F.K.

Sidbury, James B., Jr.; Cornblath, Marvin; Fisher, Jean; and House, Evelyn (Dept. of Pediatrics, Johns Hopkins Univ. School of Med., Harriet Lane Home, Johns Hopkins Hosp., and Sinai Hosp., Baltimore, Md.): GLYCOGEN IN ERYTHROCYTES OF PATIENTS WITH GLYCOGEN STORAGE DISEASE. Pediatrics *27*: 103-11, January 1961.

The ability to make an etiologic diagnosis of the various forms of glycogen storage disease from blood cells rather than liver biopsy has obvious advantages. The infant with glycogen storage disease frequently is a poor operative risk because of a bleeding tendency, hypoglycemia, hyperlipemia, and the tendency to develop marked ketosis and acidosis. The etiologic diagnosis is useful in evaluating prognosis and planning therapy. Genetically, glycogenosis shows variation in expressivity, i.e., the severity of clinical manifestations varies from one patient to another, but certain definite trends are apparent. For example, a patient with Type I glycogenosis will be stunted in growth and a problem in management until puberty. The information that the patient has Type I glycogenosis alerts the physician to the tendency to profound ketosis and acidosis, which can be precipitated by infection or other factors. Patients with muscle or liver phosphorylase, glucose-6-phosphatase defect, and Type II glycogenosis require a biopsy for definitive diagnosis. The finding of normal glycogen concentration in the erythrocytes of the patient with Type II glycogenosis was unexpected. Since increased glycogen concentration had been found in almost every tissue in this type of glycogen storage disease, it was anticipated that the erythrocytes would also reflect this generalized accumulation.

The glycogen found in the erythrocytes of patients with Type III glycogenosis was synthesized when the erythrocyte was nucleated and remains at a high level in the mature erythrocyte because the greatly reduced debrancher activity made glycogenolysis exceedingly slow. If glycogen synthesis was occurring in these erythrocytes the older cells should have a higher glycogen content than the younger cells. Such was not the case, however. One patient with amylo-1,4→1,6-transglucosidase deficiency (Type IV) was found to have a normal concentration of glycogen in the erythrocytes but the glycogen from the cellular elements of the blood was shown to be abnormal, being amylopectin-like in structure. The concentration of glycogen in the erythrocytes of Types I and II glycogenosis was found to be within the normal range. The etiologic diagnosis can be made in approximately 50 per cent of the patients with

glycogenosis by assay of the concentration and characterization of erythrocyte glycogen, thereby obviating the necessity for biopsy. The erythrocyte glycogen in Type III glycogenosis offers another parameter which may be followed in evaluating the response to therapy. W.R.K.

Spolter, Pari D.; and Harper, A. E. (Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wis.): LEUCINE-ISOLEUCINE ANTAGONISM IN THE RAT. Am. J. Physiol. *200*:513-18, March 1961.

Diets are prescribed containing 5 and 7 per cent of L-leucine which cause severe growth retardation of rats for a period of up to fourteen days. These diets are more convenient for a study of the mechanism of the growth depression than the diet containing 3 per cent of L-leucine described previously. Appetite was depressed in proportion to leucine concentration of the diet. Growth retardation of rats fed a diet containing 5 per cent of L-leucine was almost completely overcome by the addition of isoleucine and valine, and as little as 0.16 per cent of L-isoleucine + 0.15 per cent of L-valine were as effective as much higher concentrations.

The growth-retarding action of an excess of dietary L-leucine has been demonstrated with a diet in which the protein was replaced by an amino acid mixture, suggesting that the site of antagonism is not in some phase of protein digestion. No effect of excess of dietary leucine on the over-all nitrogen absorption, on nitrogen retention, or on the rate of gastric emptying was observed.

Injections of insulin counteracted the appetite-depressing effect of excess leucine in 15 to 60 per cent of rats. With the increased food intake of these rats they grew more rapidly indicating that a high intake of leucine per se is not toxic. The leucine-induced appetite depression in the remaining rats was not overcome by insulin administration with consequent death from hypoglycemia. The mechanism of the suppression of appetite by leucine and for the antagonism by isoleucine plus valine is not known but may be related to alteration in the blood amino-acid pattern. L.S.S.

Steiner, Donald F.; Rauda, Vija; and Williams, Robert H. (Dept. of Med., Univ. of Washington, Sch. of Med., Seattle, Wash.): SEVERE KETOACIDOSIS IN THE ALLOXAN DIABETIC RAT. Endocrinology *68*:809-17, May 1961.

Nonketotic alloxan diabetic rats were treated for several weeks with daily injections of Protamine Zinc Insulin. Weight gain was normal and the animals appeared healthy. Sudden withdrawal of insulin caused weight loss and fatal diabetic ketoacidosis. Livers and kidneys showed fatty infiltration. Gradual withdrawal of insulin caused weight loss and only transient ketosis with return to the nonketotic diabetic state.

Gain in body weight during insulin treatment was proportional to the dose administered. The severity of ketosis on insulin withdrawal also varied directly with the prior insulin dose suggesting that severity of acidosis was dependent upon the amount of stored body fat. H.L.W.

Stone, Daniel B. (Dept. of Int. Med., State Univ. of Iowa Hospitals, Iowa City, Ia.): A STUDY OF THE INCIDENCE AND CAUSES OF POOR CONTROL IN PATIENTS WITH DIABETES MELLITUS. Am. J. M. Sc. *241*:436-41, April 1961.

One-hundred and sixty patients with diabetes were studied to determine the degree of control and the factors which influenced this. Originally, 126 patients, or 62 per cent, were poorly regulated. Poor control could not be ascribed to inherently "unstable" diabetes. The commonest explanation for poor control was failure to adhere to diet. Many patients did not follow their diet because they did not know enough about it. Other factors which were associated with poor control were social difficulties, emotional problems, and refusal of the patient to attempt to regulate the illness.

Fifty-one of the 126 poorly controlled patients responded to the appropriate instructions and treatment by achieving good control when re-evaluated twenty-two to twenty-eight months later. Careful scrutiny of the poorly regulated patient is necessary in order that one may deal with the problem effectively. By this means, the degree of control in 160 patients was altered as follows after a period of twenty-two to twenty-eight months: 53 per cent good control, 11 per cent fair control, 36 per cent poor control as compared to initial values of 21, 17, and 62 per cent, respectively. L.S.S.

Strawitz, J. G.; Hift, H.; Ehrhardt, A.; and Cline, D. W. (Dept. of Surgery and Cardiovascular Res. Labs., Univ. of Wisconsin Med. Sch., Madison, Wis.): IRREVERSIBLE HEMORRHAGIC SHOCK IN RATS: CHANGES IN BLOOD GLUCOSE AND LIVER GLYCOGEN. Am. J. Physiol. *200*:261-63, February 1961.

Paired fed and fasted rats were subjected to a standard shock procedure consisting of a combination of tail bleeding and heart puncture with an intervening recovery period of ninety minutes. Mortality rates in the two groups did not differ significantly but the survival times were shortened in the fasted group. The fed rats survived an average of sixty-five minutes; the fasted, thirty-six minutes. The respective death rates were 58 and 81 per cent.

Following tail bleeding all animals mobilized glucose into the blood stream and developed severe hyperglycemia. In those individuals in either group which ultimately died, the blood sugar levels were further reduced following heart puncture; the rates of glucose loss were inversely proportional to survival time; the results were terminal hypoglycemias and near exhaustion of liver glycogen. In contrast, the survivors in either group, examined four hours after heart puncture, were found to be hyperglycemic and engaged in glycogen neogenesis. It is concluded that the ultimate death or survival of an animal in hemorrhagic shock is independent of its initial glucose reserves, but seems somehow to be related to its ability to maintain itself in a hyperglycemic state. L.S.S.

Swigart, Richard H.; Goldberg, Lawrence G.; and Atkinson, William B. (Dept. of Anat., Univ. of Louisville, Sch. of Med., Louisville, Ky.): GLYCOGEN IN THE UTERUS OF ALLOXAN-DIABETIC RATS. Endocrinology *68*:643-46, April 1961.

Four groups of bilaterally ovariectomized, albino rats were studied: (1) untreated, (2) estrogen treated, (3) alloxan diabetic, and (4) alloxan-diabetic estrogen treated. Uterine glycogen deposition was increased in both estrogen-treated and alloxan-diabetic rats but was highest in alloxan-diabetic rats given estrogen. Urinary bladder glycogen was unchanged throughout the experiment, while liver and muscle glycogen fell in the diabetic animal. Insulin does not appear to be necessary to the uptake of glucose by the uterine smooth muscle. Glycogen content of the uterus in the diabetic animal is related directly to the blood sugar level. H.L.W.

Unger, Roger H.; Madison, Leonard L.; and Carter, Norman W. (Veteran's Administration Hosp., University of Texas Southwestern Med. Sch., and Metabolic Clin., Parkland Memorial Hosp., Dallas, Tex.): TOLBUTAMIDE-PHENFORMIN IN KETOACIDOSIS-RESISTANT PATIENTS. J.A.M.A. *174*:2132-36, Dec. 24, 1960.

Combined tolbutamide-phenformin therapy was evaluated in ketoacidosis-resistant diabetics imperfectly controlled by tolbutamide alone. In seventy clinic patients in whom valid comparison with prior tolbutamide therapy was possible, significant reduction in the mean fasting blood sugar level was accomplished in 70 per cent after a change from tolbutamide alone to combination therapy. The antihyperglycemic effectiveness of this regimen in the tolbutamide-hyporesponsive and unresponsive groups appears to exceed by many times that of chlorpropamide, metahexamide, and phenformin alone. Tolbutamide is the drug of choice in tolbutamide-responsive diabetes; tolbutamide-phenformin combination therapy appears to be an effective and relatively safe method of treating tolbutamide-hyporesponsive and unresponsive ketoacidosis-resistant diabetes. B.F.K.

Vassalle, Mario (Depts. of Physiol. and Pharmacol., Med. Coll. of Georgia, Augusta, Ga.): ROLE OF CATECHOLAMINE RELEASE IN MORPHINE HYPERGLYCEMIA. Am. J. Physiol. *200*:530-34, March 1961.

The mechanism of morphine hyperglycemia was studied in intact and chronically adrenalectomized dogs. Adrenergic blocking agents were used to provide a further analysis of the hyperglycemic action of morphine. Venous hematocrit and plasma specific gravity were measured on the assumption that catecholamines released by morphine could elicit a contraction of the spleen. Morphine administration caused a rise in both sugar and hematocrit; after adrenalectomy, blood sugar and hematocrit showed a much smaller increase. Morphine hyperglycemia was antagonized by dihydroergotamine and dichloroisoproterenol but not by dibenamine. Since this is the pattern of epinephrine hyperglycemia it was concluded that morphine hyperglycemia is mediated by the release of catecholamines, mainly from the adrenal medulla. Atropine and artane failed to counteract morphine hyperglycemia. Thus these experiments, as far as the blood sugar is concerned, do not favor the hypothesis that morphine acts through an anticholinesterase mechanism. L.S.S.

Wasserman, Albert J.; Shiels, William S.; and Sporn, Norman I. (Med. Serv. McGuire Vet. Adm. Hosp., & Dept. of Med., Med. Coll. of Virginia, Richmond, Va.): CEREBRAL MUCORMYCOSIS. South. M. J. *54*:403-10, April 1961.

The authors review the literature of cerebral mucormycosis and place emphasis upon the typical clinical picture of the previously ill patient, usually in acidosis, most often from diabetic ketosis, who develops proptosis, ophthalmoplegia, sinusitis and a black nasal eschar. Cerebral mucormycosis is caused by true fungi of the genera Rhizopus or Mucor. There appears to be a true increase in incidence. Three cases are reported. Two with cerebral involvement died, while the other with antral, nasal and orbital infection survived. In each case the diagnosis made clinically was confirmed by histologic study.

Only two of the twenty-nine reported cases of cerebral mucormycosis have survived. Early diagnosis on a clinical basis would allow more prompt institution of therapy. The program advised is treatment of the underlying disorder with correction of acidosis, omission of antibacterial agents and adrenocortical steroids, surgical excision where feasible, and employment of amphotericin B and possibly iodides. The efficiency of this regimen is still unproved. L.S.S.

Weinges, K. F. (II. Medizinischen Klinik, Universität, München, Germany): THE EFFECT OF GLUCAGON AND INSULIN ON THE METABOLISM OF THE NONESTERIFIED FATTY ACIDS OF THE ISOLATED FAT TISSUE OF THE RAT IN VITRO. Klin. Wchnschr. *39*:293-98, March 15, 1961.

Epididymal fat tissue of young albino rats was suspended in a Krebs-Ringer-phosphate buffer solution of *p*H7.4 containing 2 per cent serum albumin and incubated in a Warburg apparatus at 37° for a period of three hours. Various doses of glucagon and insulin and different concentrations of glucose were employed. Glucagon was pretreated with cystein in order to inactivate any traces of insulin. NEFA were estimated by the method of Dole. Glucagon, in a concentration of as little as 0.004 gm./ml. caused a significant increase in NEFA production. Increase of the glucagon concentration caused a corresponding increase in NEFA. Increase of the glucose concentration in the medium inhibited this glucagon effect on NEFA production. These results seemed to be contrary to earlier observations in intact man, where the injection of glucagon was followed within one half to two hours by a decrease of the blood NEFA level. This disagreement could be explained, however, when NEFA was estimated immediately after the glucagon injection. It appeared that glucagon causes a transitory rise in the NEFA level followed by the previously observed decrease, which probably is a secondary effect due to the glucagon hyperglycemia. It appears therefore that in the intact organism too, glucagon has a direct effect upon the fat tissue. Its physiological function may therefore consist of the liberation of both glucose and NEFA from the respective depots in the liver as well as in the fat stores. (German) M.G.G.

Wiseman, Ralph, Jr.; and Baltz, Barbara E. (Radioisotope Serv., V.A. Med. Teaching Group Hosp., Memphis, Tenn.): PREVENTION OF INSULIN I^{131} ADSORPTION TO GLASS. Endocrinology *68*:354-56, February 1961.

Human serum albumin in a final concentration of 0.5 mg./ml. permits recovery of 95.8 per cent of radioinsulin from glass containers. HSA is useful in preventing losses of radio-insulin by adsorption to glass. H.L.W.

Yonet, Howard M.; and Ballard, Harold S. (Med. and Neurol. Services, Vet. Admin. Hosp., New York, N. Y.): PROLONGED SEVERE HYPOGLYCEMIA FOLLOWING TOLBUTAMIDE THERAPY FOR PARALYSIS AGITANS. New York J. Med. *61*: 1939-41, June 1, 1961.

The authors present a case of prolonged severe hypoglycemic shock following tolbutamide therapy in a nondiabetic patient with paralysis agitans. Physicians should be alerted to the fact that potentially dangerous hypoglycemic shock may occur when tolbutamide is used in the therapy of conditions other than diabetes. Should hypoglycemic reactions occur, they must be treated with the knowledge that severe hypoglycemia may persist for many hours after discontinuing the drug. C.A.R.

ORGANIZATION SECTION

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ELECTION OF OFFICERS AND COUNCILORS AT 21st ANNUAL MEETING

The Twenty-first Annual Meeting of the American Diabetes Association was held in New York City June 24-25, 1961. At the Business Session on June 25, Blair Holcomb, M.D., Portland, Oregon, was elected President for the 1961-62 organizational year to succeed Franklin B. Peck, Sr., M.D., formerly of Indianapolis.

Jerome W. Conn, M.D., Ann Arbor, Michigan, became First Vice President, and Thomas P. Sharkey, M.D., Dayton, Second Vice President. Laurentius O. Underdahl, M.D., Rochester, Minnesota, was elected Secretary, and Edwin W. Gates, M.D., Niagara Falls, Treasurer. Dr. Holcomb served in 1960-61 as First Vice President, while Dr. Conn was Second Vice President and Dr. Sharkey, Treasurer. Dr. Underdahl and Dr. Gates were Members of the Council (terms expiring 1962) All the officers will serve through the Twenty-second Annual Meeting of the Association which will be held in Chicago June 23-24, 1962, at The Conrad Hilton hotel.

At this Business Session, Joseph H. Crampton, M.D., Seattle, and Rachmiel Levine, M.D., New York, were re-elected to the Council for the three-year term expiring in 1964. Others in the same group of Councilors include Leo Goodman, M.D., Fresno; Robert C. Hardin, M.D., Iowa City; Edgar A. Haunz, M.D., Grand Forks; and Christopher J. McLoughlin, M.D., Atlanta.

At the annual session of the Assembly of Delegates, now renamed the Assembly of State Governors and Affiliate Delegates, Maurice Protas, M.D., Washington, D.C., was re-elected Chairman, and James B. Hurd, M.D., Chicago, was elected Vice Chairman. In addition to these officers the following were proposed by the Assembly to serve as members of the Council and were elected at the Annual Business Meeting: W. Bernard Yegge, M.D. (1962), Denver; Elton R. Blaisdell, M.D. (1963), Portland, Maine; and Carlisle Morse, M.D. (1964), Louisville.

ANNUAL MEETING REGISTRATION HIGHEST IN ADA HISTORY

Attendance at the Twenty-first Annual Meeting in New York reached the 800 mark, exceeding the previous high of 718 registered at the Seventeenth Annual Meeting in 1957 in the same city. Registration at the 1961 meeting and a comparison with previous years are revealed by the following figures.

		Association Members	Guest Physicians	Other Guests	Total
1961	New York	479	263	59	801
1960	Miami Beach	282	165	57	504
1959	Atlantic City	404	254	54	712
1958	San Francisco	267	165	27	459
1957	New York	493	182	43	718
1956	Chicago	406	130	29	565
1955	Atlantic City	408	137	35	580
1954	San Francisco	199	99	16	314
1953	New York	438	198	7	643
1952	Chicago	325	102	5	432
1951	Atlantic City	342	125	10	477
1950	San Francisco	162	83	6	251

Detailed information concerning the Banquet of the Twenty-first Annual Meeting and reports presented at the Business Session will be published in the Sept.-Oct. issue of DIABETES.

10th POSTGRADUATE COURSE

The American Diabetes Association will hold its Tenth Postgraduate Course in Detroit and Ann Arbor, Michigan, Jan. 17, 18 and 19, 1962. Entitled "Diabetes in Review: Clinical Conference, 1962," the Course will be presented in cooperation with the University of Michigan Medical School, Wayne State University College of Medicine and the Michigan Diabetes Association. The January 17 and 19 sessions will be held at The Statler Hilton hotel in Detroit, and the January 18 session at the University of Michigan in Ann Arbor.

The Course will be presented under the direction of the Committee on Professional Education of the American Diabetes Association. T. S. Danowski, M.D., Pittsburgh, is Chairman of the Committee and Director of the Course. Frank S. Perkin, M.D., Detroit, is Chairman of the Local Committee.

The tentative program includes the following subjects:

Wednesday morning, January 17: FUNDAMENTAL CONSIDERATIONS: "Diabetic Defects at the Cellular Level"; "Actions of Insulin and Other Hormones on Carbohydrate Metabolism of Muscle"; "Actions of Insulin in the Liver"; "Effects of Insulin and Other Hormones on Metabolism of Fat"; "Factors Regulating Insulin Release"; "Question and Answer Period by Essayists." *Wednesday afternoon, January 17*: PATHOGENESIS: "Newer Findings in Experimental Diabetes"; "Possible Variables in Genesis of Human Diabetes"; "Natural History and Course of Juvenile and Adult Diabetes"; "The Significance of the Early Recognition of the Diabetic State"; panel discussion, "Diabetes and Prediabetes, Pathogenesis and Prevention."

Thursday morning, January 18: INSULIN ASSAY—ANTAGONISM AND ACIDOSIS: "Current Applications of Insulin Assay"; "Insulin Resistance and Antagonism"; "Derangements in Diabetic Acidosis"; "Treatment of Diabetic Acidosis"; "Question and Answer Period by Essayists." *Thursday afternoon, January 18*: RELATED METABOLIC PROBLEMS: "Lipoatrophic Diabetes—A Rare but Informative Disease"; "Uses of Sulfonylurea Drugs in Hypoglycemic States"; "Glucagon—Fundamental Considerations, Diagnostic and Therapeutic Uses."

Friday morning, January 19: TREATMENT OF DIABETES MELLITUS: "Low Fat and Other Diets"; "Insulin"; panel discussion, "Problems in Management of Brittle Diabetes"; "Oral Hypoglycemic Agents"; panel discussion, "Pregnancy and Diabetes."

Richard J. Bing, M.D., Chairman, Department of Medicine, Wayne State University College of Medicine, will deliver an address on "Myocardial Metabolism in Diabetes" at the Friday Luncheon Meeting.

Friday afternoon, January 19: COMPLICATIONS OF DIABETES MELLITUS: "Vascular Disease in Diabetes"; "Specificity of Vascular Lesions in Diabetes"; "Pathology of Eye in the Diabetic"; panel, "Complications of Diabetes Mellitus"; "Present and Future Research Problems in Diabetes Mellitus."

Registration Fees are $40 for Association members and $75 for nonmembers. All inquiries pertaining to the Course should be directed to the American Diabetes Association, 1 East 45th Street, New York 17, N. Y.

ADA RESEARCH FELLOWSHIP DEADLINE

Nov. 15, 1961, is the deadline for receipt of applications for the academic year of July 1, 1962—June 30, 1963. At least three Research Fellowships will be awarded by the Committee on Research and Fellowships. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

1962 LILLY AWARD

The sixth Lilly Award will be given at the Twenty-second Annual Meeting of the American Diabetes Association June 23-24, 1962, in Chicago. The award is supported by Eli Lilly and Company and consists of $1,000 and a medal. The following stipulations govern the contest.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before December 1 of the year preceding the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to December 1 of the year preceding the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

DIABETES WEEK IS NOVEMBER 12-18

The week before Thanksgiving (November 12-18) will be observed as Diabetes Week. Members of the Association and readers of the Journal are urged to participate in this important Public Education and Detection program through their local Diabetes Association or County or State Medical Society.

ADA AMENDS BYLAWS

The Bylaws of the American Diabetes Association were amended rather extensively at the Twenty-first Annual Meeting, after several years of consideration by the Committee on Policies, the Committee on Constitution and Bylaws, and the Council. The primary purpose of the revision is to provide Affiliate Associations greater representation and voice in the activities of the national organization. The revised Bylaws will be published at a subsequent date and an announcement of their publication will be printed in a future issue of the Journal.

NEW MEMBERS

The following were elected as of June 1, and July 1, 1961:

Active

Alabama	
McGehee, John McD.	Mobile
Parker, Leslie L.	Andalusia
California	
Green, Donald E.	Beverly Hills
Florida	
Glassberg, J. E.	Miami Beach
Ireland, T. L.	Pompano Beach
Georgia	
Newton, M. C.	Macon
Pavlovsky, W. M.	Atlanta
Illinois	
Murphy, J. W.	Taylorville
Quinn, T. W.	Chicago
Indiana	
Maha, George E.	Indianapolis
Massachusetts	
Bleicher, S. J.	Boston
Litonjua, A. D.	Boston
Millman, Max	Springfield
Yerganian, George	Boston
Michigan	
Grishkoff, M. A.	Detroit
Lowrey, George H.	Ann Arbor
New York	
New, Maria I.	New York
North Carolina	
Blackburn, J. T.	Winston-Salem
Burt, Richard L.	Winston-Salem
Seals, D. H.	Sylva
Ohio	
Wyler, Carl I.	Cincinnati
Virginia	
Gant, Frank L.	Richmond

Associate

South Carolina	
Chrisanthis, Miss Anna	Charleston

Other Countries

Canada	
Lovatt, G. E.	London, Ont.
Philippines	
Villegas-Cinco, A. R.	Manila

NEWS OF AFFILIATE ASSOCIATIONS

The CONNECTICUT DIABETES ASSOCIATION held its annual meeting in Hamden April 26, 1961. Edward Tolstoi, M.D., Professor of Clinical Medicine at Cornell University Medical College, spoke on "Is Coronary Heart Disease More Prevalent Among Diabetics?"

The DALLAS DIABETES ASSOCIATION concluded a very successful 1960-61 scientific series in May. During the year four guest speakers addressed the Clinical Society: Alfred Bongiovanni, M.D., Philadelphia, spoke about recent advances in the field of intersex; Arnold Lazarow, M.D., Minneapolis, discussed some of the pathological physiology associated with the development of complications of diabetes; Robert H. Williams, M.D., Seattle, talked about new advances related to the field of diabetes; and Vincent Dole, M.D., New York, talked about metabolism of adipose tissue and its relationship to diabetes. The Clinical Society has invited approximately twenty guests, resident physicians and fellows in metabolic diseases, to each of its dinner meetings.

The NEW ENGLAND DIABETES ASSOCIATION held its annual meeting in Cambridge on May 25, 1961. A "Symposium on Peripheral Vascular Disease" was presented with the following participants: Robert F. Bradley, M.D., Edward Edwards, M.D., Edward Frank, M.D., Philip M. LeCompte, M.D., and Frank C. Wheelock, M.D. At the winter clinical meeting held in Boston on January 17, Paul E. Lacy, M.D., St. Louis, discussed "The Physiology of Islets of Langerhans as Studied with Electron Microscopy, Immunochemical and Microchemical Technics."

The NEW YORK DIABETES ASSOCIATION will hold the Ninth Annual All-day Symposium on Dec. 1, 1961, in the Grand Ballroom of the Hotel Pierre in New York City. Entitled "Insulin and Its Use," with Gerald Friedman, M.D., as Chairman, it will feature the following subjects: "Chemistry of Insulin," "Mechanism of Action of Insulin," "Insulin Antibodies," "Electron Microscopy of Islet Cells," "Effect of Insulin on Protein Metabolism," "The History of the Discovery of Insulin," "The Clinical Impact of Insulin," and "The Effect of Insulin on the Liver." Charles H. Best, M.D., Toronto, who with the late Sir Frederick G. Banting isolated the life-saving hormone insulin, will participate in the Symposium.

Physicians, medical students and other professional personnel interested in diabetes are welcome to attend the Symposium. There is no registration fee. Advance registration is required and may be made by calling the New York Diabetes Asso-

ciation, 104 East 40th Street, New York 16, N. Y. A subscription luncheon will be held at the Hotel Pierre. Admission to the meetings and luncheon will be by ticket only.

The New York Diabetes Association has established a new program for diabetics, The Vocational and Counseling Service. The primary aims of the Service, which is available without charge, are twofold: to help diabetics with their educational and career problems, and to carry on a continuing educational program on the employability of diabetics.

The ROCHESTER REGIONAL DIABETES ASSOCIATION held its annual meeting on May 18, at which William Lotspeich, M.D., Professor of Physiology, University of Rochester School of Medicine and Dentistry, spoke on "Phlorhizin and Diabetes."

NEWS NOTES

ANNUAL MEETING OF THE AMERICAN DIETETIC ASSOCIATION

The forty-fourth Annual Meeting of The American Dietetic Association will take place in St. Louis, Missouri, Oct. 24-27, 1961. Sessions for the first three days will be held at the Kiel Auditorium where an extensive exhibition of food service equipment and food products will be on display. On Friday, the last day, sessions will be held at the Sheraton-Jefferson, the headquarters hotel.

Among those who have been invited to participate in a discussion on diabetes are these members of the American Diabetes Association: Herbert Pollack, M.D., New York (current concepts of fat in diabetic diets); Harvey C. Knowles, Jr., M.D., Cincinnati (a five-year follow-up of children with diabetes); and Lawrence E. Hinkle, Jr., M.D., New York (effects of customs, emotions, and behavior on diabetes mellitus). W. Stanley Hartroft, M.D., St. Louis, will discuss modification of the American diet in relation to atherosclerosis.

COMMUNICATION INSTITUTE FORMS NEW SCIENTIFIC COUNCIL

The Institute for Advancement of Medical Communication, with headquarters in New York City, has formed a scientific council to guide its program of research, development and training aimed at improving communication among biomedical scientists and health science practitioners. The council consists of twenty scientists and educators, who represent many of the disciplines and specialties contributing to and using the expanding pool of biomedical information. The Institute also has opened a Washington branch at 1028 Connecticut Ave., N.W., Washington, D.C.

ADA MEMBERS PARTICIPATE IN GERIATRICS PROGRAM

The American Geriatrics Society held its eighteenth annual meeting in New York June 22-24, 1961, at which the following members of the American Diabetes Association participated: Edward L. Bortz, M.D., Philadelphia, Presiding Officer June 22 for a panel discussion entitled "Energy Supply and Maintenance," with Laurance W. Kinsell, M.D., Oakland, as moderator, and Edgar S. Gordon, M.D., Madison, Wis., as a participant. Albert E. Renold, M.D., Boston, presented "Adipose Tissue as a Site of Hormonal and Nutritional Regulation," during a panel discussion entitled "The Interrelation of Carbohydrate and Lipid Metabolism." Thomas H. McGavack, M.D., Martinsburg, West Virginia, was moderator for a panel discussion entitled "Endocrine Regulatory Mechanisms as People Age," Russell M. Wilder, Jr., M.D., Topeka, spoke on "Neuroendocrine Relationships," Raymond V. Randall, M.D., Rochester, Minnesota, on "The Pituitary," and Reginald A. Shipley, M.D., Cleveland, on "The Gonads." Dr. Bortz, President of the American Geriatrics Society, and Past President of the American Medical Association, delivered an address on June 24.

NATIONAL VITAMIN FOUNDATION AWARDS GRANTS-IN-AID

The Board of Governors of the National Vitamin Foundation has approved six new grants-in-aid for studies on the effect of vitamin A on mucin formation of the uterine epithelium; of drug toxicity in vitamin-deficient animals; for the expansion of long-term vitamin research; for metabolic studies in patients with pyridoxine responsive anemia; for studies of tocopherol and fatty acid relationships in tissues; and of the function and metabolism of vitamin A.

PERSONALS

GEORGE J. HAMWI, M.D., Columbus, will present a paper on "Endocrine Causes of Hypertension" on Wednesday morning, Sept. 20, 1961, during Postgraduate Course No. 1 of The American College of Physicians. Entitled "Changing Concepts of Cardiopulmonary Disease," the course will be offered Sept. 18-23, 1961, at University Hospital, The Ohio State University Health Center, Columbus, Ohio.

LEO WADE, M.D., New York, has been appointed Vice President for Administration and Member of the Sloan-Kettering Institute for Cancer Research. Dr. Wade, who assumed his new post on July 1, was formerly Medical Director of Esso Standard, Division of Humble Oil and Refining Company.

MICHAEL G. WOHL, M.D., Philadelphia, Chairman of the Committee on Nutrition and Metabolism of the Philadelphia County Medical Society, will preside at a luncheon meeting during a symposium entitled "Nutrition and Oral Health," which will be presented Sept. 24, 1961, at the Evans Dental Institute in Philadelphia.

NECROLOGY

DOUGLAS H. AITKEN, Wilmington, Delaware, born September 24, 1917.

MICHAEL E. BRODSKY, Bridgeport, Connecticut, born April 21, 1901.

AUGUSTINE E. CONROY, Lowell, Massachusetts, born October 12, 1891.

JOHN B. KARR, Chicago, Illinois, born March 15, 1897.